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ONCOLOGY

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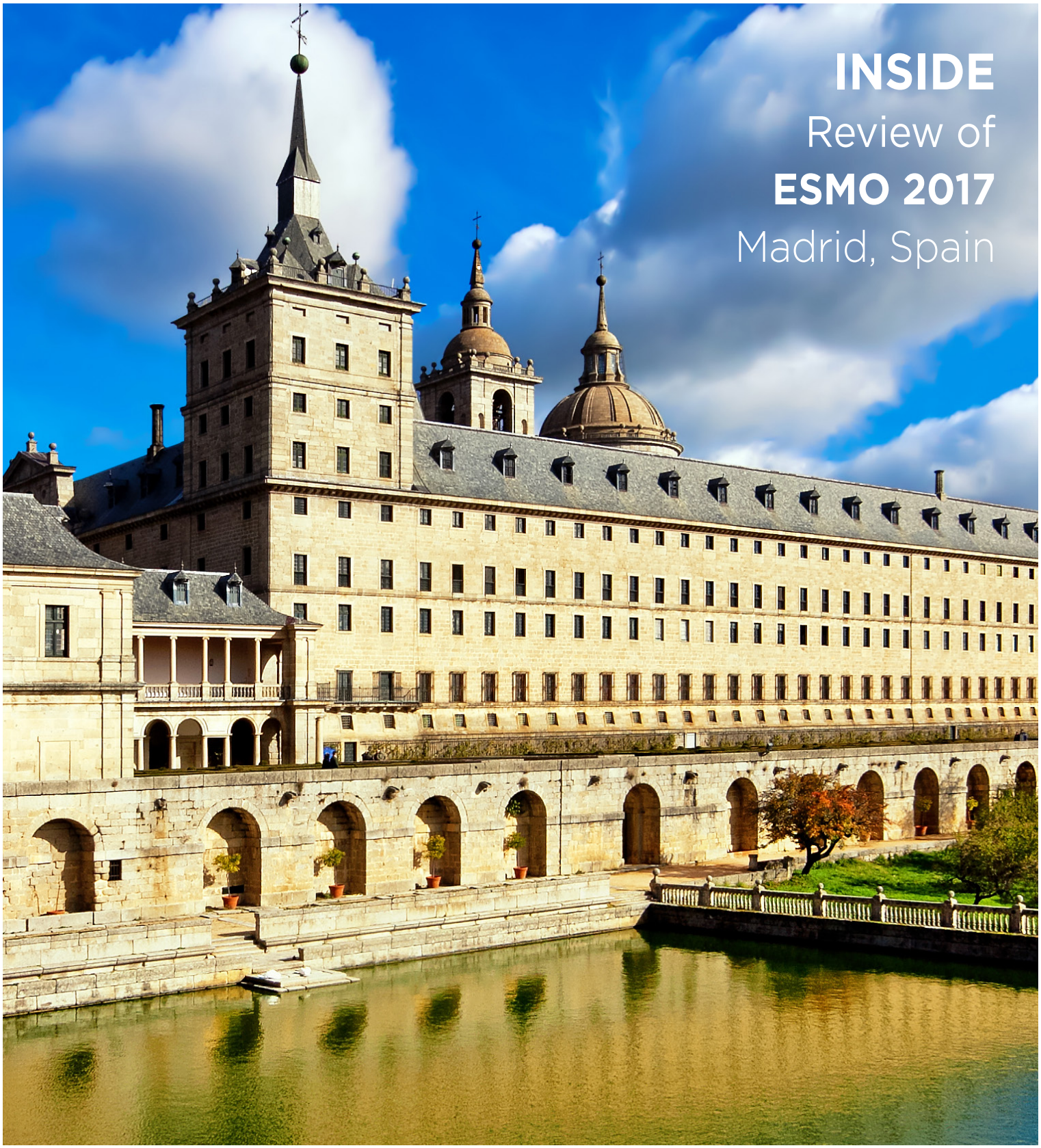

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ONCOLOGY

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ONCOLOGY 5.1
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EUROPEAN UNION
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Welcome

A very warm welcome to the 2017 edition of *EMJ Oncology*. Within this eJournal you will find a myriad of the latest exciting advances within the field, including featured reviews from the European Society for Medical Oncology (ESMO) 2017 Annual Congress. The event, which took place in Madrid, Spain, over the 8th–12th September, 2017, accepted a monumental 1,736 abstracts for presentation. With almost 24,000 attendees at the event, rest assured you will find some truly exhilarating topics for debate enclosed within. A selection of the abstracts presented over the 5-day event are presented inside, alongside interviews from our highly acclaimed Editorial Board and exceptional peer-reviewed articles.

> "... there is a topic within to spark the imagination of every reader."

The reduced ability to work experienced by cancer survivors, and insights into the perceptions of oncology patients affecting their likelihood to participate in clinical trials, are just two examples of the topics you can expect to find within the Congress Review section of *EMJ Oncology 5.1*, all taken from press releases reported at the ESMO Congress. Interviews with our Editorial Board give you a glimpse into their careers within oncology, including cases they have found particularly interesting and their views on the current challenges within the field. In our Abstract Review section, Archer et al. summarise their intriguing presentation from the ESMO Congress 2017, focussing on the differences between the normal and malignant prostate. In addition, Ferroni et al. present their abstract regarding the challenges for venous thromboembolism risk assessments, and provide a novel approach for developing the topic using a machine-learning approach based on multiple kernel learning. There are many more thrilling abstracts to be enjoyed within.

The whole EMJ team are delighted to bring you a vast array of peer-reviewed articles. From this edition's Editor's Pick, wherein Huertas and Lechuga deliver an extensive review on epigenetic biomarkers and the use of optical biosensors for analysis, to a discussion from Chen et al. on the latest chemotherapy agents available for the treatment of germ cell tumours in paediatric, adolescent, and young adult patients, there is a topic within to spark the imagination of every reader.

We hope you find this year's *EMJ Oncology* eJournal as captivating as we have, and that the content covered inspires you to strive for further progression within the field. The EMJ team are already eagerly awaiting next year's ESMO Annual Congress, which is guaranteed to deliver even greater discoveries, promote the collaboration of experts from all around the world, and stimulate intense discussion at every turn.

Kind regards,

Spencer Gore

Director, European Medical Journal

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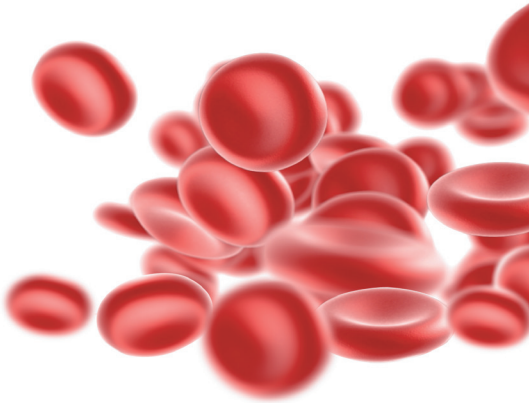

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Foreword

Dr Luís Costa

Hospital de Santa Maria, Lisbon, Portugal

Dear Colleagues,

I am delighted to welcome you to this issue of *EMJ Oncology 5.1*, home to the latest developments in cancer care and treatment from around the world. Within, you will find a wide range of exciting peer-reviewed articles, as well as an insightful review of the European Society for Medical Oncology (ESMO) Congress, held this year in Madrid, Spain.

As we have come to expect from such a prestigious organisation, this year's ESMO Congress was bigger and better than ever before, with almost 24,000 attendees. The congress provided an exceptional platform for collaboration and innovation, bringing the full force of the international oncology community to bear against a common enemy: the myriad of diseases encompassed within the term cancer. With a huge array of symposia, lectures, poster sessions, and exhibitions, the event showcased the very best in oncology research, the highlights of which are presented throughout this journal.

> "The world of oncology is rapidly changing and I feel this journal captures some of its most crucial developments."

In addition to the excellent Congress Review, this journal also contains a selection of important peer-reviewed articles seeking to change clinical practice. Chen et al. tackle the issues surrounding chemotherapy for extracranial germ cell tumours in paediatric, adolescent, and young adult patients: a demographic in dire need of improvement in care. Niccolai et al. review the dual role of immune cells in the onset and progression of pancreatic cancer, Zouk and Batra guide us through the latest developments in malignant pleural mesothelioma diagnostics and therapeutics, and Lin and Xi discuss the role of ^{18}F-fluorodeoxyglucose positron emission tomography as an aid in oesophageal cancer management. Morlacco et al. engage with the, thus far, unanswered questions surrounding adjunctive treatments following prostatectomy, highlighting the importance of adjunctive therapy in cancer treatment. Finally, Huertas and Lechuga explore the field of optical biosensors for the detection of epigenetic biomarkers, showing them to be cheap and effective, and calling them the future of cancer diagnosis in this issue's Editor's Pick.

This is merely a taste of all that is contained within the journal and I am proud to present *EMJ Oncology 5.1* to all of you, my colleagues. I hope that the material within will prompt exciting discussion and help guide your clinical practice. The world of oncology is rapidly changing and I feel this journal captures some of its most crucial developments. Please enjoy.

Yours sincerely,

Luís Costa

Professor of Medicine and Principal Investigator, Instituto de Medicina Molecular, University of Lisbon; Hospital de Santa Maria, Lisbon, Portugal

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AML is swarming with challenges

Strikingly heterogeneous at diagnosis, acute myeloid leukaemia (AML) is a complex disease that has a rapidly evolving and distinct pattern of molecular abnormalities. Many important kinases and proteins are involved, with up to two-thirds of AML patients having a FLT3 (ITD or TKD) and/or NPM1 mutation.[1,2]

Progress has been made in supportive treatment as well as in understanding the prognostic importance of oncogenic drivers underlying the onset of AML. Although there have been many incremental advances over several decades, challenges remain.[2]

Novartis Oncology is dedicated to the international research effort to find new options for AML patients worldwide.

For more information, please visit:
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FLT3, FMS-like tyrosine kinase 3; ITD, internal tandem duplication; NPM1, nucleophosmin 1; TKD, tyrosine kinase domain.

References: 1. Patel JP, Gönen M, Figueroa ME, et al. Prognostic relevance of integrated genetic profiling in acute myeloid leukemia. *N Engl J Med.* 2012;366:1079-1089. **2.** Ferrara F, Schiffer CA. Acute myeloid leukaemia in adults. *Lancet.* 2013;381:484-495.

October 2016 G-PKC-1149410

ESMO ANNUAL CONGRESS 2017

IFEMA-FERIA DE MADRID,
MADRID, SPAIN
8TH–12TH SEPTEMBER 2017

Welcome to the European Medical Journal review of the Annual Meeting of the European Society for Medical Oncology

Citation: EMJ Oncol. 2017;5[1]:12-29. Congress Review.

After a 3-year period, the historic Spanish capital, Madrid, once again welcomed the European Society for Medical Oncology (ESMO) Annual Meeting, from the 8th–12th September 2017. As always, collaboration was a huge focus of this year's ESMO Congress, which was, for the first time, hosted in partnership with the European Association for Cancer Research (EACR), bringing together an unprecedented wealth of expertise for visiting delegates. Nearly 24,000 people from >130 countries were in attendance for this revolutionary congress, which was described by ESMO President Prof Fortunato Ciardiello as: "the most important oncology congress in the history of Europe". ESMO membership itself continues to grow, with around 17,000 members, nearly 40% of whom are from outside of Europe, making the congress a truly international event. "We are a global home for everyone that is an oncology professional, with the same goal, the same dream, the same ambition: to cure cancer patients," said Prof Ciardiello.

This congress featured the announcement of new ESMO guidelines, aiming to give clinicians all the tools they need to make the right therapeutic and diagnostic choices, as well as outlining, for the first time, the therapeutic use of biosimilars. The opening ceremony also featured a selection of prestigious awards; Miguel Martin, President of the Spanish Society of Medical Oncology (SEOM), received the ESMO Award for his outstanding contribution to promoting the speciality of medical oncology; José Baselga received the Lifetime Achievement Award for his impressive work in breast cancer drug development; Alberto Bardelli was recognised with the Translational Research Award for his work on liquid biopsy for colorectal cancer; and Frances Shepherd was bestowed the Women for Oncology Award in the prestigious Women for Oncology session later in the week.

As always, the ESMO programme was large, varied, and interactive. With around 40% of ESMO's members being <40 years of age, there was a strong focus on young oncologists, offering an excellent educational platform to provide them with the latest and most important research. As oncology research continues to advance, so too does an increased need for a multidisciplinary approach for care and diagnosis, and as a result the congress programme had specific sessions

dedicated to each branch of cancer, with an emphasis on interdisciplinary collaboration. New light was also shed on management, aftercare, and communication with cancer survivors, who are all too frequently overlooked in the heat of the ongoing battle against cancer. With keynote lectures, late breaking abstracts, satellite symposia, and numerous interactive sessions, this event provided a myriad of opportunities for oncologists and beyond. The programme also featured three presidential sessions, showcasing the most crucial, practice-changing revelations at this year's congress, many of which are summarised in the following section of our journal. Echoed in every aspect of the ESMO programme were Prof Ciardiello's words from the opening ceremony: "We are united, all together, with one dream: to destroy the word cancer."

> "We are a global home for everyone that is an oncology professional, with the same goal, the same dream, the same ambition: to cure cancer patients."

You can find the main highlights from this impressive congress in the following Congress Review section, including research on gastric, cervical, oesophageal, and other cancers, as well as on patient communication and crucial data from cancer survivors. Whether you were lucky enough to attend the congress or if you missed out, we hope that you find this section not only interesting and enjoyable, but instructional and influential in your day-to-day clinical practice. The passion on display at this year's ESMO congress was incomparable and we are certain it will be emulated and even surpassed next year, when the event moves to one of Germany's most beautiful cities, Munich. As always, we look forward to seeing you there.

Hybrid Minimally Invasive Oesophagectomy for Oesophageal Cancer

A COMPARISON between hybrid minimally invasive oesophagectomy (HMIO) and transthoracic open oesophagectomy (OO) as the surgical options in patients with oesophageal cancer revealed that HMIO should become the standard operating procedure for these patients. These results, from the Phase III MIRO trial, were presented in a ESMO press release dated 5th September 2017. Speaking about the findings of the trial, Prof Ulrich Güller, Kantonsspital St. Gallen, St. Gallen, Switzerland, announced: "This represents an extremely important, well-designed, and well-conducted study demonstrating that HMIO is an oncologically sound procedure and significantly reduces postoperative morbidity. Based on these results, the HMIO should become the new standard operating procedure for patients with mid and low oesophageal cancer."

> "Based on these results, the HMIO should become the new standard operating procedure for patients with mid and low oesophageal cancer."

In this open-label, randomised controlled trial, which was conducted at 13 study centres, 207 patients with resectable cancers of the middle or lower third of the oesophagus were randomly assigned on a 1:1 basis to receive either HMIO or transthoracic OO (103 patients in the HMIO group). It was found that significantly fewer patients in the HMIO study arm had major postoperative morbidity in comparison with the transthoracic OO study arm (35.9% versus 64.4%; odds ratio: 0.31; 95% confidence interval [CI]: 0.18–0.55; p<0.001). Furthermore, the HMIO presented with a reduced incidence of major pulmonary complications (17.7% versus 30.1%; p=0.037).

While these results demonstrated the reduced surgical trauma associated with HMIO, it was also important to consider the long-term efficacy of the treatment. At a median of 48.8 month follow-up, it was found that overall survival was 67.0% in the HMIO study arm (95% CI: 57.0–75.2%), which compared to 54.8% in the transthoracic OO study arm (95% CI: 44.8–63.8%; p=0.054).

While the study authors noted the survival difference was not statistically significant between the study arm, they suggested it was of great clinical relevance.

Pembrolizumab Improves Results for Pretreated Metastatic Gastric Cancer Patients

METASTATIC gastric cancer patients, previously treated with chemotherapy, have shown encouraging responses to pembrolizumab treatment, according to data from the KEYNOTE-059 trial presented at this year's ESMO congress and reported in a ESMO press release dated 8th September 2017. With expected survival of metastatic gastric cancer patients currently <1 year and a distinct lack of new drugs having been developed in the last decade, patients have been left wanting for adequate treatment.

> "These results have set the stage for a larger follow-up study which is already enrolling patients."

This Phase II KEYNOTE-059 trial, one of the largest studies to investigate immunotherapy in recurrent or metastatic gastric cancer, subdivided patients into three cohorts. The 1st (n=259) included patients with metastatic gastric cancer who received the programmed death 1 inhibitor, pembrolizumab, after pretreatment with two or more chemotherapy lines. The 2nd (n=25) assessed newly diagnosed metastatic gastric cancer patients receiving combination therapy of pembrolizumab and chemotherapy. The 3rd(n=31) consisted of newly diagnosed metastatic gastric cancer patients, treated solely with pembrolizumab.

Cohorts 1 and 2 included both PD-L1 positive (PD-L1+) and negative (PD-L1-) patients, whereas Cohort 3 included only PD-L1+ patients. Cohort 1 had an overall response rate (ORR) of 12%, but further analysis revealed ORR for PD-L1+ and PD-L1- patients were 16% and 6%, respectively. Cohort 2 showed a similar PD-L1 genotype ORR distribution, with the ORR for the cohort measuring 60% and PD-L1+ and PD-L1- ORR of 73% and 38%, respectively. Cohort 3 had an ORR of 26%. Grade 3–5 treatment-related adverse events were recorded in 18% of Cohort 1 and 3% of participants discontinued treatment as a result.

This study showed PD-L1+ patients responded best to pembrolizumab, especially in combination with chemotherapy. Both combination therapy and pembrolizumab alone were safe and showed promising results. Lead author Dr Zev Wainberg, co-director of the Gastrointestinal Oncology Programme, UCLA, Los Angeles, California, USA, said: "These results have set the stage for a larger follow-up study which is already enrolling patients."

Dr Ian Chau, Royal Marsden Hospital, London and Surrey, UK, explained: "Unlike with chemotherapy, toxicities from immunotherapy tend to occur later on. We need to await longer-term results from an ongoing clinical trial in an earlier line of treatment to know the full impact of this drug." Dr Chau concluded that further research is needed to refine the PD-L1 biomarker and search for other biomarkers to indicate which patients would respond best to therapies, as well as to gain further information on quality of life.

Docetaxel-Based Triplet Therapy Shows Superiority in Gastric Cancer

DOCETAXEL-based triplet therapy has shown superiority over standard therapy in the treatment of patients with resectable oesophago-gastric cancer, results from a recent study reported in a ESMO press release dated 8th September 2017 confirmed. With survival in this population poor (roughly 25% with surgery, increased to 36% with the addition of a perioperative regimen of epirubicin, cisplatin, and infused fluorouracil [ECF]), these results bring a great deal of optimism to clinicians and patients.

The FLOT4 study is the first trial to show an improvement in outcomes from another well-established therapy in this patient population. In the study, 716 patients were randomised to perioperative docetaxel, oxaliplatin, and fluorouracil/leucovorin (FLOT) or ECF. The primary endpoint was overall survival, and FLOT was demonstrated to outperform ECF in this and other endpoints for efficacy, including curative resection rates and progression-free survival.

> “FLOT will be the best backbone of chemotherapy that we can use in this setting.”

The team performed multivariate, subgroup, and sensitivity analyses to consolidate the results, which showed that the relative effect of FLOT could be seen in all patient subgroups. It was associated with fewer cases of progressive disease during and after preoperative therapy (1% versus 5%; p<0.001), more R0 resections (84% versus 77%; p=0.011), a higher number of pT0 and pT1 tumours (25% versus 15%; p=0.001), longer progression-free survival (30 versus 18 months; p=0.001), and better overall survival (50 versus 35 months; p=0.012).

In their concluding remarks, the authors noted that FLOT was of benefit even in patients who were elderly (>70 years of age), if their tumour was small, had a nodal negative status, or had a signet cell component, suggesting that these factors need no longer dissuade clinicians from considering perioperative therapy. Prof Michel Ducreux, Gastrointestinal Unit, Institut Gustave Roussy, Villejuif, France, commented: “FLOT will be the best backbone of chemotherapy that we can use in this setting. A step forward would be to try to improve the results by adding targeted therapies or immune checkpoint inhibitors. It would also be interesting to know if the FLOT regimen shows different levels of effectiveness in the four molecular biological subgroups of gastric cancer.”

Young Adult Cancer Survivors Experience Reduced Work Ability

DISCOVERIES from the NOR-CAYACS study presented at the ESMO congress 2017 have highlighted how young cancer survivors

suffer from late side effects of their cancer treatment, affecting their career progression and development, as reported in a ESMO press release dated 9th September 2017.

The study focussed on cancer survivors who were diagnosed between the ages of 19 and 39 years, with cancer types including melanoma, colorectal cancer, breast cancer Stages I–III, non-Hodgkin lymphoma, and leukaemia. This particular age range is usually when foundations are laid for establishing careers and families; therefore, cancer diagnoses within this age bracket can negatively affect this progression.

The study comprised a questionnaire regarding the late side effects of cancer treatment and work status. It required the 1,198 participants involved to score themselves from 0–10 on the Work Ability Index, with 10 being the highest work ability. The results showed that low scores related to several factors, including, but not limited to, lymphoedema, female sex, depression, low level of education, and reduced physical quality of life and self-reported health. Those survivors who had suffered non-Hodgkin lymphoma were more likely to have a reduced work ability compared to those who suffered melanoma. Interestingly, the intensity of treatment received was not correlated with work ability.

Lead author Dr Cecilie Kiserud, National Advisory Unit for Late Effects After Cancer Treatment, Oslo University Hospital, Oslo, Norway, said: "Greater awareness is needed about the fact that cancer survivors may be less able to work after treatment because of the late effects they might experience." Another study presented at the same congress found that 67% of healthcare professionals treating young adult cancer patients were unable to provide proper care due to a lack of access to specialist care centres. By highlighting the complications cancer survivors experience after treatment, as well as the gaps in current patient care, improvements can be made which will provide these patients with the best possible chance to lead normal lives.

"Greater awareness is needed about the fact that cancer survivors may be less able to work after treatment because of the late effects they might experience."

Further Analysis of Treatments for Prostate Cancer

TWO NEW TREATMENTS for prostate cancer were found to have no statistically significant difference in terms of overall survival, according to late breaking results from the STAMPEDE trial, which were announced at the ESMO congress and reported in a ESMO press release dated 8th September 2017. At present, the treatment of choice for individuals with high-risk locally advanced or metastatic prostate cancer is long-term hormone therapy and has been for >70 years. However, earlier results from the STAMPEDE trial have demonstrated superior survival rates compared to standard of care for docetaxel (hazard ratio [HR]: 0.78) and abiraterone acetate plus prednisolone (AAP) (HR: 0.63).

In the context of these previous results, researchers utilised prospectively collected data from the STAMPEDE trial to facilitate a direct comparison between the docetaxel treatment arm and the AAP arm. In total, the comparison focussed on 189 patients from the docetaxel arm and 377 from the AAP arm. All patients were recruited between November 2011 and March 2013 and additionally received standard of care androgen deprivation therapy, with some patients also being treated with radiotherapy. While the researchers noted that this comparison was underpowered, they pointed out that this was the only data available that compared the two therapeutic options.

The study's primary outcome was overall survival. The estimate for this was a HR of 1.16. It was found that there was no statistically significant difference between the treatment arms. Estimates of the early treatment outcome measures (failure-free survival and progression-free survival) did favour APP (HR: 0.51 and 0.65, respectively). However, there were no statistically significant differences between the treatment arms in regard to the late outcome measures of freedom from symptomatic skeletal events and freedom from metastatic progression, although the estimates did favour AAP.

> "This study suggests that starting with either drug is acceptable and choice may depend on therapy."

Speaking about the results, STAMPEDE's chief investigator, Prof Nicholas D. James, University of Birmingham and Queen Elizabeth Hospital, Birmingham, UK, explained: "This study suggests that starting with either drug is acceptable and choice may depend on therapy." It was also commented that toxicities associated with the treatments and patient preferences and characteristics will influence the choice of therapy. Looking to the future, it was noted that there was a need to evaluate cardiovascular outcomes for patients receiving AAP. Furthermore, ongoing trials are evaluating a combination of docetaxel and new hormone therapies, including AAP.

Psycho-social Impact of Chemotherapy

SOCIO-PSYCHOLOGICAL factors have overtaken physical side effects, such as nausea and vomiting, as the most significant impacts of chemotherapy for cancer patients, according to a ESMO press release dated 9th September 2017 and results presented at the 2017 ESMO congress.

> "The results show that there might be a gap between what doctors think is important or disturbing patients, and what patients really think."

It is well known that side effects of chemotherapy seriously impact patients' lives and managing these effects is a constant concern for doctors. There have been regular assessments of these side effects since 1983, but this study has shown that perceptions of chemotherapy side effects for breast and ovarian cancer change over time, as well as through the treatment course. Study author Dr Beyhan Ataseven, Kliniken Essen Mitte Evang, Huyssens-Stiftung, Essen, Germany, felt it was important to conduct this study to update the interview format and collect new data.

Differing from previous research, this study focussed solely on breast and ovarian cancer and involved three interviews, before, during, and after treatment, producing longitudinal data. A total of 141 patients preparing to begin

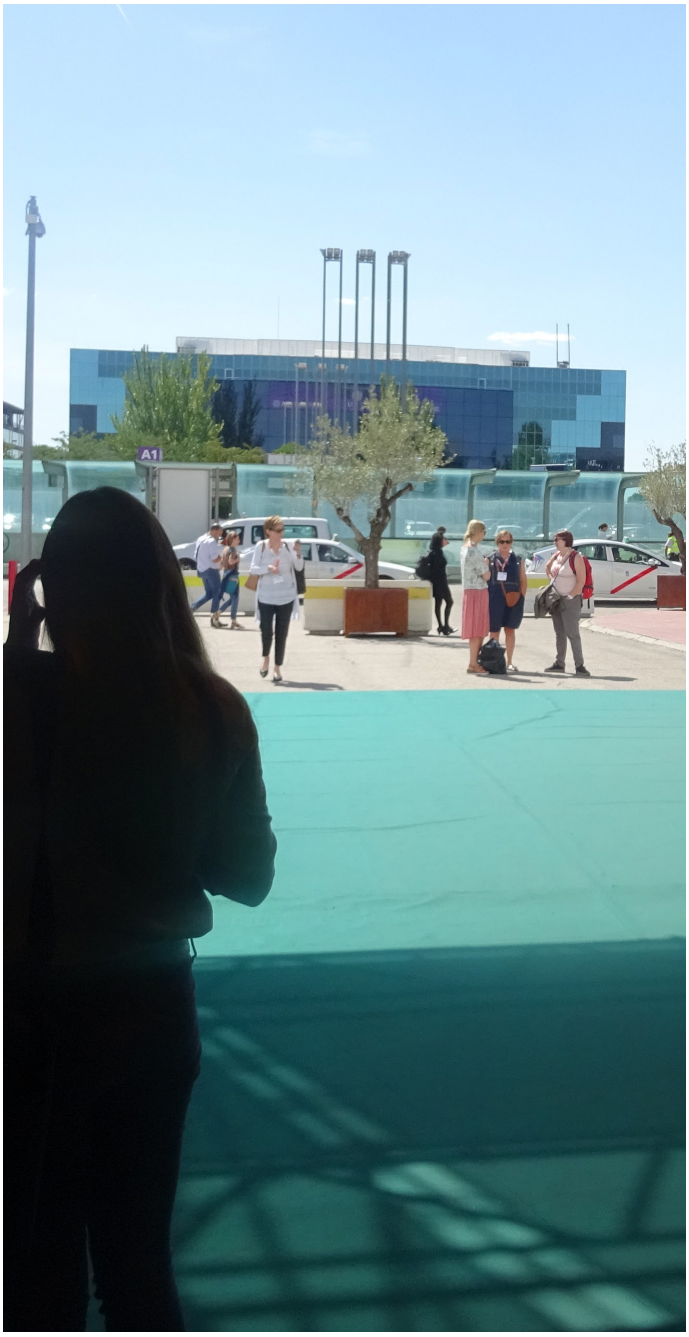
or undergoing chemotherapy were included in the study; all participants were presented with two groups of cards, one card group included physical and the other, non-physical side effects of chemotherapy. Patients were asked to select what they believed to be the five most burdensome symptoms in each group and rank them according to importance for each group separately. Of the 10 selected side effects, they were then asked to select the five most significant of all the side effects and then rank them according to overall importance.

It was found that physical side effects (nausea and vomiting) were no longer significant problems for patients, explained, perhaps, by modern medications for these side effects being very effective. Dr Ataseven further explained: "Hair loss is still a persistent, unsolved issue that particularly affects patients at the start of their treatment. As time passes and patients get used to this, however, their concerns evolve and other side effects become more significant." She went on to explain that throughout the course of treatment the most difficult side effects for patients are sleep disorders along with anxiety about the effects of the illness on partners or family, which consistently remains a top issue.

Dr Karin Jordan, Department of Medicine, University of Heidelberg, Heidelberg, Germany, commented: "The results show that there might be a gap between what doctors think is important or disturbing patients, and what patients really think. Physical, psychological, social, and spiritual support is needed at every stage of the disease." Further research is still needed to investigate other cancer types.

Positive Response to New Palliative Care Tool

SPECIALIST palliative care has been shown to improve prognosis, amongst other benefits, for certain subgroups of oncology patients, a ESMO press release dated 9th September 2017 reports. Now, a new tool named "Triggers" could help clinicians to identify which patients would benefit from this referral. Triggers was developed by the London Cancer Alliance and the pilot study is being carried out at The Royal Marsden NHS Foundation, London, UK, a ESMO Designated Centre of Integrated Oncology and Palliative Care.



> “The goal is for the tool to become standard and easy for anyone on a patient’s primary care team to use, for us, the next step will be to expand into other tumour groups.”

The Foundation’s lung oncology clinic was the first to use the Triggers tool and, in the first 4 months of use, 84% (97 out of 115) of patients deemed ‘eligible’ were screened within 2 months of their first appointment. A prospective, longitudinal, observational service evaluation was carried out to assess the level of satisfaction from patients who were referred and to describe the needs of palliative care patients who were reviewed using the Triggers tool.

Commenting on the preliminary results, Dr Jayne Wood, The Royal Marsden and Royal Brompton Hospitals, London, UK: “We found that 75% of the patients reviewed triggered positive on ≥1 of the tool items. Of the ‘Trigger positive’ cohort, whose needs were then assessed by a palliative care team, 97% were identified as having at least a moderate need for specialist palliative care, even though 81% of them were still functioning well, ranking in the top two scores on the scale used to assess how a disease affects a patient’s daily living abilities.” She added: “This tells us that we are addressing a real need, and that the tool is picking up a group of patients who have a real potential to benefit from referral to specialist palliative care. The goal is for the tool to become standard and easy for anyone on a patient’s primary care team to use, for us, the next step will be to expand into other tumour groups.” One of the patients who was referred to the palliative care team commented: “They have helped me with medication, which has given me more energy, visited me at home, and have been able to advise me about different symptoms. I definitely feel that I can call them if I need them.”

Chemoradiation: The Best Treatment for Advanced Cervical Cancer

CHEMORADIATION has been deemed the best standard treatment option currently available for locally advanced cervical cancer. Recent trial results, presented at the ESMO 2017 congress, have shown that the alternative treatment, neoadjuvant chemotherapy followed by surgery, showed no improvement in disease-free survival

rates when directly compared to chemoradiation alone, as reported in a ESMO press release dated 10th September 2017.

> “Patients who received chemotherapy followed by surgery were less likely to be alive and disease-free at 5 years than those who received standard treatment with chemoradiation.”

Chemotherapy has long remained the standard treatment option for locally advanced cervical cancer, but with survival rates still not at 100%, researchers have been encouraged to find potential improvements in treatment options. This 14-year study included 633 patients with Stage IB2, IIA, or IIB squamous cell cervical cancer. The patients were randomly assigned to two treatment groups: neoadjuvant chemotherapy (paclitaxel and carboplatin) with radical hysterectomy afterwards, or chemoradiation alone (standard pelvic radiation plus cisplatin). The study’s primary endpoint was disease-free survival without relapse or death from the cancer.

The results showed that after a median of 58.5 months, the endpoint was achieved in 30% of the chemotherapy with surgery patient group, and 23% of the chemoradiation therapy group. After 5 years, however, this was significantly improved; the endpoint was achieved in 69.3% of the chemotherapy plus surgery patient group, and 76.7% of the chemoradiation group (p=0.038).

First author Dr Sudeep Gupta, Tata Memorial Centre, Mumbai, India, said: “Patients who received chemotherapy followed by surgery were less likely to be alive and disease-free at 5 years than those who received standard treatment with chemoradiation.” He concluded that chemoradiation should remain the treatment of choice, except in situations where this modern therapy may not be available, such as in developing countries. In situations where chemoradiation is not available, neoadjuvant chemotherapy combined with surgery may be the best option. A 2nd trial is currently underway assessing the same two treatment options, with hopes that the additional results will provide more concrete evidence as to the best treatment option.

Cancer Risk Factor in Postmenopausal Women

DISTRIBUTION of body fat is a more significant risk factor for the development of cancer than body weight alone in postmenopausal women, according to the results of a study reported in a ESMO press release dated 10th September 2017. One of the study’s investigators, Line Mærsk Staunstrup, Nordic Bioscience and ProScion, Herlev, Denmark, spoke about the impact of this finding. She stated: “When assessing cancer risk, BMI and fat percentage may not be adequate measures as they fail to assess the distribution of fat mass. Avoiding central obesity may confer the best protection.”

This finding was a result of the Prospective Epidemiologic Risk Factor study, intended to facilitate a superior understanding of age-related diseases in Danish women post menopause. In this study, 5,855 women with a mean age of 71 years had their body fat and body fat composition assessed at baseline by means of dual-energy X-ray absorptiometry scans. The women were followed over a period of 12 years.

The study researchers utilised data from national cancer registries to determine that, over the follow-up period, there had been 811 solid cancer incidences in the cohort. While neither BMI nor fat percentage showed a significant association with cancer diagnosis, a high ratio of abdominal fat to peripheral fat did demonstrate an association (hazard ratio [HR]: 130; 95% confidence interval [CI]: 1.11–1.52; p<0.001). When the association was considered by specific cancer type it was found that only lung and gastrointestinal cancers were associated with a high ratio of abdominal to peripheral fat (HR: 1.68; 95% CI: 1.12–2.53; p<0.05; and HR: 1.34; 95% CI: 1.0–1.8; p<0.05, respectively). After controlling for the factors of older age, smoking, and receipt of hormone replacement therapy, fat ratio was found to be an independent risk factor.

This finding offers opportunities for detecting those at a greater risk of cancer development. Furthermore, it was speculated that a potential intervention alongside fat loss through dietary changes and exercise could be the use of diabetes drugs to lower the effect of insulin and hence reduce visceral and abdominal fat accumulation.

> “When assessing cancer risk, BMI and fat percentage may not be adequate measures as they fail to assess the distribution of fat mass.”

Rucaparib Improves Progression-Free Survival

RUCAPARIB therapy improved progression-free survival (PFS) in *BRCA* positive recurrent ovarian cancer by 77%, according to the ARIEL3 trial results presented at the ESMO 2017 congress and reported in a ESMO press release dated 8th September 2017. Ovarian cancer has notoriously poor survival rates, with most cases presenting at advanced stages and 80% recurring after first-line treatment. Even when patients are treated with platinum-based chemotherapy, which generally yields a good response, most relapse again and eventually die of the disease.

Rucaparib is a poly ADP-ribose polymerase (PARP) inhibitor that blocks DNA repair in *BRCA* positive cells and, as a result, kills these *BRCA*-mutated cells. Nearly 20% of ovarian cancer patients express *BRCA* mutations and thus would be suitable for PARP inhibitor treatment. Other ovarian cancer patients, such as those with a high degree of genomic loss of heterozygosity (LOH) and those who respond well to platinum-based chemotherapy, would also be suitable for rucaparib treatment.

The ARIEL3 trial included 564 high-grade ovarian cancer patients who previously responded to platinum-based chemotherapy as either a second or third-line treatment and were randomised (2:1) for rucaparib and

placebo. The primary endpoint was measured in three groups: Group 1: *BRCA* mutant; Group 2: *BRCA* mutant or BRCA wild-type with high LOH; and Group 3: intention to treat (the entire study population).

> “Rucaparib is clearly an exemplary member of this exciting class of drugs that can be used to treat women with recurrent ovarian cancer.”

Results showed a statistically significant improvement in PFS for those treated with rucaparib. PFS increased from 5.4 months to 16.6, 13.6, and 10.8 months in Groups 1, 2, and 3, respectively, with respective hazard ratios of 0.23, 0.32, and 0.36. The improvement in PFS was statistically significant for all subgroups, with the greatest improvement in those with *BRCA* mutations who indicated a 77% increase. Patients with high LOH had a greater PFS improvement than those with low LOH, but both subgroups responded significantly better than the placebo group. Due to poor side effects, 13% of patients withdrew from treatment, but overall rucaparib was well tolerated.

Prof Jonathan Ledermann, University College London Cancer Institute, London, UK, commented: “PARP inhibitors are the biggest development in ovarian cancer therapy since the introduction of platinum drugs in the late 1970s and early 1980s. Rucaparib is clearly an exemplary member of this exciting class of drugs that can be used to treat women with recurrent ovarian cancer.”

Dr Andrés Poveda, Gynaecological Cancer Clinic, Oncology Foundation Institute, Valencia, Spain, described the results as achieving: “a huge decrease in the risk of relapse with rucaparib”, and went on to conclude: “personalised medicine has arrived in high grade serious ovarian cancer. Further studies are needed to identify predictive biomarkers of response to PARP inhibitors.”

Optimistic Results from the LORELEI Trial

PATIENTS with oestrogen receptor positive and HER2-negative early breast cancer may benefit from treatment with taselisib, a ESMO press release dated 8th September 2017 reports. It is the first trial to note a significant increase in objective response rate (ORR) in this subgroup of patients following treatment with a phosphoinositide 3-kinase selective inhibitor.

The team enrolled 334 postmenopausal patients with early operable oestrogen receptor positive, HER2-negative breast cancer who had had tissue analysis for *PIK3CA* mutant cancer cells. The study co-primary endpoints were ORR (tumour size measured using magnetic resonance imaging [MRI]) and pathologic complete response rate (detection of cancer cells at the site following surgery). Participants were randomised to receive letrozole alongside either a placebo (n=168) or taselisib (n=166) for 16 weeks, with a view to reducing tumour size prior to surgery.

Reduction in taselisib dose or discontinuation occurred in 11.4% and 10.8%, respectively, and Grade 3 and 4 adverse effects were noted: gastrointestinal disorders (7.8%), infections (4.8%), skin or subcutaneous tissue disorders (4.8%), vascular disorders (3.6%), and metabolism and nutrition disorders (3.6%). One sudden death was recorded in the taselisib group, but it was considered to be unrelated to the drug.

> We were able to detect a reduction in tumour size after only 16 weeks of treatment...

The results, however, were overwhelmingly positive. ORR was higher in the taselisib group (50.0%) compared to those who received placebo (39.3%) (odds ratio: 1.55; 95% confidence interval: 1.00–2.38; p=0.049). No significant difference was detected between the pathologic complete response groups. It was noted that in patients whose tissue analysis detected *PIK3CA* mutation (n=152), taselisib demonstrated even greater efficacy; 56.2% showed ORR compared to 38.0% in the placebo group (odds ratio: 2.03; 95% confidence interval: 1.06–3.88; p=0.033).

Commenting on the study, Dr Cristina Saura, Breast Cancer and Melanoma Group, Vall d'Hebron Institute of Oncology (VHIO), Barcelona, Spain, explained: "We were able to detect a reduction in tumour size after only 16 weeks of treatment, compared to patients who received letrozole plus placebo. Any decrease in tumour measurements is something positive for patients because this means the drug has had activity against their tumour in a short period of time," adding: "For me, the main message is that even though all patients seems to derive some benefit from taselisib, those who had this mutation seemed to derive more benefit."

Misguided Perceptions Cause Reduced Clinical Trial Participation

CLINICAL trials are deemed important to oncology patients, but recent results presented at the ESMO 2017 congress showed that they have difficulty understanding the methodologies behind them, as reported in a ESMO press release dated 10th September 2017. The nationwide study, which took place in Ireland, discovered these results while providing questionnaires to a total of 1,090 adult oncology patients from 14 different oncology centres, regarding new cancer treatment development trials.

New cancer treatments are constantly being researched and developed, and clinical trials play a huge role in determining their success. Despite this, and even with oncology patients acknowledging their importance, the actual number of patients participating is extremely low, alongside the annual accrual to clinical trials, thought to be around 3–5%.

> Doctors have a responsibility to properly inform their patients in this regard, because they are the ones patients trust the most.

The questionnaire included statements about clinical trials, with patients required to answer "True", "False", or "Don't Know". One important finding was that 73% of patients who had not participated in a trial before were unaware that the treatments were distributed within a trial completely at random, with no one knowing which treatment will prove best, known as clinical equipoise. Over half (56.5%) of patients thought their doctor would know the best treatment, and 60.9% thought their doctor would give them that supposedly best treatment. In addition, 22% of patients thought clinical trials were only used when standard treatments had failed.

Dr Catherine Kelly, Mater Misericordiae University Hospital, Dublin, Ireland, said: "Doctors have a responsibility to properly inform their patients in this regard, because

they are the ones patients trust the most." With more patients understanding the concepts of clinical trials, they may begin to participate more, resulting in a much greater knowledge regarding new cancer treatments.

As the median age of patients in this study was 60 years, future research will be targeted towards younger patient groups, and also across different tumour classifications, where information and care is accessed and pursued differently, to ensure patients are informed as effectively as possible.

Validity of ESMO 'Magnitude of Benefit' Scale for Orphan Drugs

ORPHAN DRUGS can be legitimately graded by the ESMO Magnitude of Clinical Benefit Scale (ESMO-MCBS), according to the results of recent research presented at the ESMO congress and reported in a ESMO press release dated 10th September 2017. The ESMO-MCBS has been designed to assess new therapeutic treatments of cancer and assign them a relative ranking in regard to their expected clinically meaningful benefit. In an era of limited resources, such a tool is important to ensure the maximum healthcare benefit is obtained from money spent.

The study researchers examined clinical trials that had resulted in U.S. Food and Drug Administration (FDA) approval and applied the ESMO-MCBS to the drugs. Furthermore, the trials for orphan and non-orphan drugs were compared in terms of their characteristics, such as methodology. Using the Drugs@FDA website, the researchers found 137 studies, of which 109 were randomised controlled trials; as a result of these trials, 63 drugs were approved between January 2006 and December 2016 for 118 indications. Forty-six percent of these drugs were classified as orphan drugs.

Upon comparing the characteristics of randomised controlled trials for non-orphan drugs with orphan drugs, the researchers noted that trials for non-orphan drugs tended to have a greater sample size (median: 687 versus 369; p=0.001), were more commonly randomised (86% versus 73%; p=0.047), and had a greater tendency to assess overall survival as opposed to intermediate endpoints (51% versus 71%; p=0.01). Additionally, a greater percentage of non-orphan drug trials evaluated endocrine therapy or experimental cytotoxic therapy as compared to targeted therapy (21% versus 8%; p=0.005).

On applying the ESMO-MCBS, the researchers determined that 29% of orphan drugs with FDA approval for palliative care met the threshold for meaningful benefit. The corresponding figure for non-orphan drugs was 27%. Speaking about the results, the lead author, Ms Consolación Molto Valiente, Department of Medical Oncology, Hospital de la Santa Creu i Sant Pau, Barcelona, Spain, stated: "The ESMO-MCBS is applicable in most situations where randomised controlled trials are available, including those supporting approval for drugs granted orphan designation." Ms Valiente did note that the application of the ESMO-MCBS is more limited in the case of orphan drugs because >25% of drug approvals are supported by single-arm studies. It is hoped that demonstrating the applicability of the ESMO-MCBS for orphan drugs represents another step on ESMO's path towards sustainable cancer care.

> "The ESMO-MCBS is applicable in most situations where randomised controlled trials are available, including those supporting approval for drugs granted orphan designation."

Rare Cancers Treated Using Drug Rediscovery by DNA Sequencing

PATIENTS with rare cancers with currently no standard treatment could be eligible to receive therapies that have already been applied to more common cancers that carry the same genetic mutation, according to results presented at the ESMO 2017 congress and reported in a ESMO press release dated 8th September 2017.

The Centre for Personalised Cancer Treatment (CPCT) includes 40 Dutch hospitals and collects and analyses metastatic cancer biopsies by whole genome sequencing, creating a database which now includes 2,000 individuals treated for different types of cancer. Principal study investigator Prof Emilie Voest, Netherlands Cancer Institute, Amsterdam, Netherlands, explained that by sequencing the genome of so many patients, commonalities were found between tumours and mutations; one example was *ERBB2* mutations, which are usually only screened for in breast cancer but are also present in other tumours.

Once similarities were identified, the Drug Rediscovery Protocol was introduced, currently including 19 drugs. Since the launch of the trial in 2016, >250 cases have been submitted for review, with ˜70 eligible patients. "We have preclinical evidence and case reports suggesting that certain drugs, which patients with a given genetic aberration and a certain type of cancer are sensitive to, could equally be active in patients with the same mutation in other tumour groups. However, we also know that the tissue background is extremely important; that is why we create study cohorts not just according to genetic mutation, but also according to the specific tumour type," Prof Voest explained.

Prof Voest went on to explain the two-stage treatment efficacy analysis: "If in Stage 1, the 1st group of eight patients with the same tumour type and genetic mutation responds to the treatment, we expand the cohort to 24 patients in Stage 2 to get a stronger indication of the clinical benefit." Clinical benefit was defined as either complete remission, partial response (>50% tumour shrinkage), or disease stability for >16 weeks. Clinical benefit was observed in 37% of trial participants; 6 out of 20 study cohorts progressed to Stage 2. "We have seen real success with several anticancer drugs, including immunotherapy, a poly ADP-ribose polymerase inhibitor, and an antibody combination," Prof Voest commented.

This process of drug rediscovery using DNA sequencing is a novel technique and could ultimately save health services money by reducing the number of new drugs having to be bought for cancers that could be treated with pre-existing drugs. The cost benefit will have to be assessed further; however, this study is definite proof of concept, and will inspire further research.

> "...certain drugs, which patients with a given genetic aberration and a certain type of cancer are sensitive to, could equally be active in patients with the same mutation in other tumour groups."

Mohammad Akheel

Consultant Head and Neck Oncosurgeon, CHL-CBCC Cancer Centre, Indore, India.

Q: We understand that you specialise in otolaryngology. Could you tell us whether a specific person or event inspired you to choose this career path?

A: I had a dream to be a head and neck onco-surgeon like Dr Jatin P. Shah (Memorial Sloan Kettering Cancer Center [MSKCC], New York City, New York, USA) even before I chose my fellowship programme. The field of oncology is challenging and we need to balance the benefits and risks for these patients. I struggle, but try my best, to give a new life to my cancer patients. I want to treat as many patients as I can with my surgical skills and knowledge to improve the quality of life of cancer patients.

Q: In 2016, you published a book titled 'Surgeon's Knife: Head & Neck Incisions'. Could you tell us more about this publication and what inspired you to write this?

A: Early in my fellowship days, I was asked by my guide to operate on a case of fungating carcinoma of buccal mucosa. I was a little sceptical about the planning of the incision and I ended up taking a wrong one. My guide went wild and asked me to get descrubbed and learn about the flaps' viability and incisions. This particular incident motivated me to write a book exclusively on head and neck incisions and I am very thankful to my publisher in India for making it a valuable and presentable piece of information. I am currently writing my next book on head and neck oncology.

Q: Your book talks about the importance of aesthetic results following surgery to the head or neck, particularly with regards to the face. Do you think aesthetic considerations are overlooked in other aspects of oncology?

A: According to my experience, gone are the days of radical onco-surgery. There are many cancer centres that still follow old incisions and techniques of radical surgery. I feel that to improve the quality of life of these cancer patients, aesthetic onco-surgery needs to be practised without compromising the oncologic principles for further prosthetic rehabilitation of the patient; thereby improving the quality of life socially, physically, and psychologically. "The face is the temple of the mind". The microstructure of the face needs to be reconstructed well following onco-surgery to maintain the self-esteem of the patient.

Q: Could you please provide us with a brief insight of your current area of research?

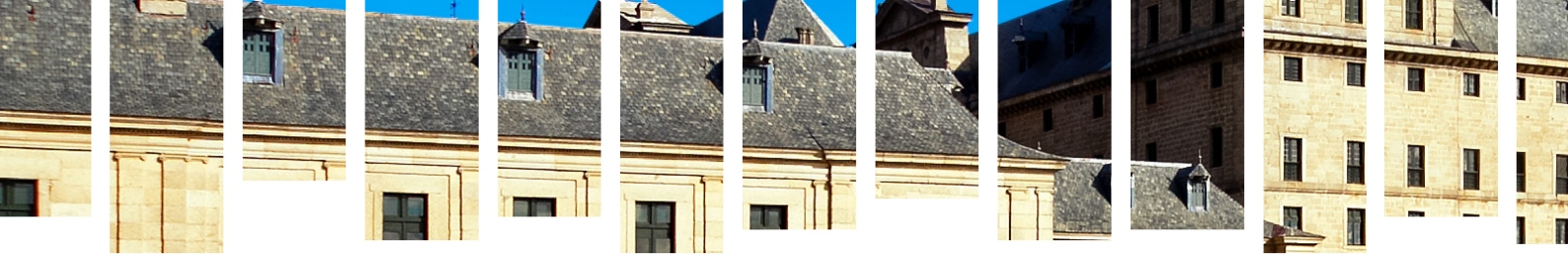
A: Currently, I am doing my Doctorate of Philosophy in oral and maxillofacial surgery and my research is in head and neck cancers in relation to their prognostic parameters.

Q: Could you tell us about the Comprehensive Blood and Cancer Centre (CHL-CBCC) and your roles and responsibilities within it?

A: The CHL-CBCC is a dedicated cancer centre with surgical, medical, and radiation oncology under one roof. It has 15–20 head and neck cancer patients every month. I was appointed as an associate onco-surgeon and my duties are to perform and assist in surgeries when required and to take care of the intensive care unit (ICU) and wards.

Q: What is the most interesting or rewarding case on which you have worked?

A: There are many. Most of the cases I get are fungating T3-T4 lesions. I perform my cases with the upmost determination. In restricted mouth opening cases of tongue and floor of mouth, I perform paramedian access mandibulotomies. There are many young patients who report with T4 lesion with huge para-mandibular soft tissue involvement, necrotic nodes for combined mandibulectomy, and neck dissection operation



(COMMONDO) surgery with pectoralis major myocutaneous flaps. Each case is interesting and rewarding, with challenges inspiring me to improve even more.

Q: What do you think about the position of cancer research and treatment in India? What would you like to see change over the next 10 years?

A: There are so many trials being conducted and lots of research happening to break the 5-year barrier. I think immunotherapy and targeted therapy shall take over in the coming 10 years, rather than conventional chemotherapy. Image-guided radiation therapy and RapidArc® are good recent advancements in radiation. But to me, the role of surgery will remain the gold standard.

Q: Are there any conditions or complications that are increasing in prevalence in your field? How will this impact the field in the future?

A: Surgical complications are common and do respond well over a period of time. We need to do something about the side effects of radiation therapy. Tobacco chewing and other illicit substances need to be strictly banned in India.

Q: What do you consider to be the biggest challenges that the field of oncology faces, both in India and globally?

A: Tobacco chewing, low socio-economic status, malnutrition, and no public health awareness are some of the reasons that India has so many head and neck cancer patients. There is a need to establish a centralised system for protocol of cancer management with tumour board meetings because every cancer centre, or every onco-surgeon, follows their own personal protocol.

Q: What advice would you like to pass on to oncologists hoping to specialise in otolaryngology?

A: Determination and hard work are two things which I believe will lead to excellence. Dedication to aesthetic onco-surgery will give better results and quality of life to your patients. Learn well, perform well, and do well. God has given us the power of healing hands, utilise it wisely. Have a great day and my best wishes to the budding onco-surgeons.

> “I feel that to improve the quality of life of these cancer patients, aesthetic onco-surgery needs to be practised without compromising the oncologic principles for further prosthetic rehabilitation of the patient...”

Klaus Seiersen

Medical physicist, Danish Centre for Particle Therapy, Aarhus University Hospital, Aarhus, Denmark.

Q: Firstly, could you please tell us a little bit about your career path; what made you become interested in medical physics?

A: I have had a quite varied career path. I did my PhD and a short postdoc at the University of Aarhus, Aarhus, Denmark, studying molecular ions colliding with free electrons at the large ASTRID accelerator facility. After this, I switched to teaching and outreach projects for a while. First, I worked on implementing an online database with physics demonstration experiments for teachers, and then I spent some time travelling Europe and Asia with a ‘physics show’. We did physics demonstration experiments at science fairs, town squares, and various public events.

As the project money ran out, I started looking for other job opportunities and found an opening as a medical physicist. I had friends working in the field, and they recommended it, so I applied. I got the job and have now enjoyed working in this both challenging and rewarding field since 2006.

> “My experience with public teaching started with basic physics...”

Q: We understand that the Danish Centre for Particle Therapy (DCPT) is currently under construction at Aarhus University Hospital and is expecting its first patients in 2018. Could you tell us more about the overall vision for the centre and how you hope it will influence oncology in Denmark, and potentially beyond?

A: We have a clear vision of making a world-class treatment and research centre. The DCPT will have three gantries for patient treatments and a fixed beam room primarily for research. Aarhus has a well-established radiobiological research group, and we hope to expand this field into the proton realm.

Most of our patients will enter research protocols, and although the first proton therapy treatment was delivered in the USA more than 60 years ago, we still consider it very much an experimental treatment. Being a public healthcare centre, we will not struggle with the same insurance barriers that private centres have faced all over the world, and we do not expect problems recruiting patients from all over the country.

Protons will, of course, be a completely new treatment modality to consider for oncologists at the existing seven photon centres in Denmark, but it is an important part of the implementation process of DCPT to involve all Danish Multidisciplinary Cancer Groups, and also the cancer centres in, for example, designing protocols and, in general, building the necessary competences for proton therapy on a national level. We are already organising national video conferences where proton cases are discussed, and we are having workshops on everything from research protocols to practical treatment planning issues.

Q: What advantages does proton therapy have that makes it more attractive than alternative treatment options? Also, are there any drawbacks of proton therapy?

A: The dosimetric properties of protons are completely different from standard photon X-ray radiotherapy. Basically, whereas photons deposit the maximum dose right under the skin, continue straight through the tumour, and exit the other side of the patient, protons will stop inside the patient and deliver the maximum dose right inside the tumour.

The main advantage of this is that proton therapy has a potential to treat the patient with far fewer side effects. When the toxicity is reduced, perhaps you can even escalate the dose to the tumour, which, in theory, can result in higher local control and higher survival rates.

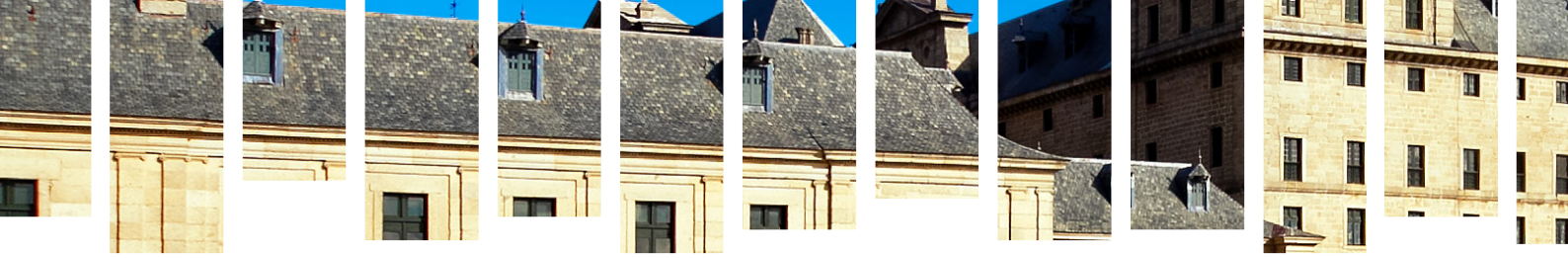
However, the dosimetric advantage of protons is also its biggest weakness as a therapy. When the protons simply stop, you are facing the risk of 'missing the target with the utmost precision'. The entire treatment is based on your ability to stop the protons at the exact right position, but there are a lot of uncertainties to take into consideration: computed tomography (CT) scan noise and artefacts, patient and organ motion, proton beam energy variations, etc.

Also, the biology of proton therapy is not similar to X-ray therapy. Biologically speaking, cells react differently when you deposit 1 joule of proton energy compared to 1 joule of X-ray energy. The difference is of the order of 10%, but the exact factor depends on factors such as depth in tissue, tissue type, and beam energy. This introduces further uncertainties into the treatment and emphasises the importance of biological research.

Q: You are a member of the Paediatric Radiation Oncology Society (PROS), the only internationally operating society for paediatric radiation oncology. What are the society's aims and what does your role within the organisation entail?

A: The objectives of PROS are to set a worldwide standard of excellence of radiotherapy for children and to provide a forum for knowledge exchange between professionals. One of the main activities is organising the PROS congress, which brings together all experts in the field.

PROS was founded by oncologists, but, recognising the roles of other allied professionals like medical



physicists, two seats of the Executive Committee of PROS are reserved for non-oncologists. I am presently serving my second term on the committee, with a Canadian radiation nurse serving as the other allied professional.

My contribution has been to establish an international network of physicists working with paediatric radiotherapy. The network now comprises almost 100 members from 33 different countries. We have used this network to establish a medical physics symposium at the last PROS congress in New York City, New York, USA, something which was very positively evaluated by the attending oncologists.

Another focus area of PROS is education and support for low and lower-middle income countries. Earlier this year, this brought me to Cairo in Egypt, where we did a 3-day workshop on paediatric radiotherapy.

Q: What is it that makes paediatric malignancies such a challenge for radiation oncologists?

A: Paediatric malignancies are a great challenge due to their rarity, the great variety of histological types, and the complexity of treatment techniques. Many oncologists might only see relatively few paediatric patients during an entire career, and it is quite difficult to gain experience on any specific histological subgroup. It is therefore particularly important to continuously exchange knowledge across centres and to set up international research protocols recruiting patients from many different countries.

From a radiation treatment perspective, the challenges are complex. Children are more sensitive to radiation than adults, and patients will have many more years after successful cure to suffer from the possible treatment side effects. Children are also more sensitive to other types of effects than adults, including bone growth inhibition, which can have both a cosmetic influence and also more serious effects on quality of life.

Most notably, the ionising radiation used for curing the patients might also result in a secondary radiation-induced malignancy that can appear decades after treatment. It is therefore important to limit the total dose of radiation used during treatment. Proton therapy is a possible alternative treatment option in that respect.

Q: You founded the Danish Physics Show in Aarhus, bringing scientific education to schools and the public at large. How important do you believe the role of extracurricular education in terms of cancer prevention and nurturing the next generation of oncologists is?

A: I believe it is important for all science professionals to communicate to the public about work being done at universities, healthcare centres, and research institutions. Teaching is not for everyone, but you should at least have some, who are good at it, devoting some time to it.

My experience with public teaching started with basic physics, and this was initiated because the faculty of science for many years suffered under a falling number of new students, which meant that they could not supply the number of candidates for high tech jobs that the society required. This launched an extensive outreach programme of which I was a part.

In Denmark, both university education and healthcare is free of charge, and for that reason we also have an obligation to tell society about how, and on what, tax money is spent. At our hospital, we have had several activities where we try to inform the public about cancer and radiotherapy. We feel the public should know about the advanced and complicated treatments the society can offer cancer patients. After all, one-third of Danes will get a cancer diagnosis in their lifetime, so almost everyone will sooner or later either receive radiotherapy or have close friends or family members receiving radiotherapy.

People should know that Danish cancer treatment is world-class; and not just people receiving it, but also journalists writing about it, politicians funding it, and of course young doctors still considering their future speciality.

> “I believe it is important for all science professionals to communicate to the public about work being done at universities, healthcare centres, and research institutions.”

Q: According to the NORDCAN project, Denmark has the worst cancer incidence of all the Nordic countries. Is there anything you would like to see done on a national level to rectify this?

A: In Denmark, we have had a large focus on the survival rate of Danish cancer patients, which has in general been lower than in other Nordic countries. This has been addressed by politicians for many years now. A national cancer plan from 2000 resulted in a massive capacity upgrade of Danish radiotherapy, and the 2014 ESTRO-HERO project documented that Denmark now has the largest number of treatment linear accelerators per capita in Europe. Also, the Danish ‘cancer packages’ from 2007 introduced accelerated diagnostic courses for patients suspected of cancer, something now being copied by Sweden and Norway. By 2016 the cancer survival rates were comparable to other Nordic countries.

It is true that cancer incidence is still higher in Denmark and political focus is now switching towards prevention. We have much higher tobacco and alcohol consumption than our neighbours, so I think there are many political tools to improve the numbers. It is my personal belief that all sales of tobacco products will be completely banned within the next 20 years.

Q: You have been an avid blogger for many years, regularly updating your blog at www.scienceblog.dk. What can you tell us about the role of social media in medical science, particularly in niche fields such as yours?

A: More and more researchers are now using social media to disseminate knowledge, and I find this development very important. We are living in a time where simple anecdotes are circulated on the internet as actual facts, and it is more important than ever to counter misinformation with solid research. For example, this is the case for the anti-vaccination lobby; following their recommendations can have serious consequences to patients.

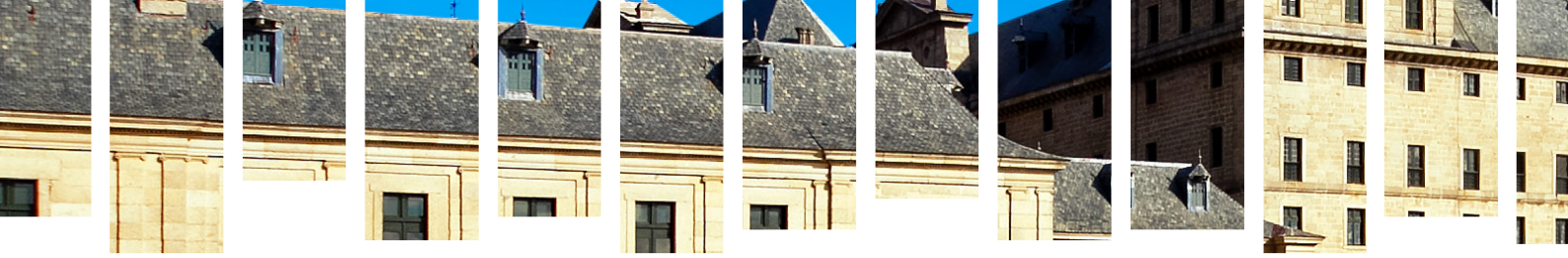
The different electronic media have different potential and reach. About two-thirds of all Danes are active on Facebook, while perhaps <10% of Danes are on Twitter. Though Twitter is not used by that many people, it is the preferred media of many politicians, journalists, opinion makers, and, in particular, people using social media for professional reasons. To reach the layperson with your message, Facebook is an effective media. I use the science blog for more elaborate texts, which are then advertised on Facebook, from which traffic is directed towards the blog.

My previous experience with social media has allowed me to get involved in the Twitter account of the DCPT, which is mainly updated by three medical physicists. It has been a new experience for the Communications Department to have clinical professionals, and not journalists, updating department social media, but we have succeeded in gaining a large number of followers, many of which are themselves involved in particle therapy. This allows us to have a direct communication channel to our international colleagues interested in regular updates on our progress.

Q: In your opinion, how successful are events such as the European Society for Medical Oncology (ESMO) congress at disseminating new research and techniques amongst the medical community?

A: Professional congresses are essential in any scientist’s career. I have never participated in a scientific congress without bringing home new knowledge. I usually prepare specific topics I want to follow up on during the congress, but often the main take-home messages come from topics I did not plan to focus on during the meeting.

Also, the networking character of congresses is quite important. To meet fellow physicists and oncologists from other centres establishes



contacts that are useful in future work. The PROS congress is, for example, well known for building relationships. The congress is much smaller than the ESMO congress, with only 150 participants. Some like to call it a 'family reunion', but the result is that paediatric radiation professionals have a tight network and on a routine basis exchange views on difficult clinical cases, which is so very important for the field.

Q: Finally, what advice would you give to a young researcher looking to get into this fascinating field?

A: I have worked with both basic research in molecular physics and with teaching of fundamental physical concepts to a wide audience, but going into medical physics has given me a completely new dimension to my work.

My advice is simple: No matter what you do, never choose a career based on salary, geography, or job opportunities. Make sure you choose to work with something you are passionate about. Passion will make you spend the time and effort necessary to become an expert in your field, and this will almost automatically lead you onto an interesting career path.

I have had a passion for physics. This brought me into exciting work with research and teaching and finally to treating paediatric cancer patients. I had never planned for this when arriving at the university, but the passion for physics brought me to this very rewarding field, where my knowledge of physics can mean the difference between a cure and treatment failure.

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DEXAMETHASONE CONTRIBUTES TO THE SIDE EFFECTS IN GLIOBLASTOMA TREATMENT

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Keywords: Glioblastoma (GBM), dexamethasone (DXM), side effect, anticancer therapy, proteoglycan.

Glioblastoma (GBM) is the most common and malignant primary brain tumour and is characterised by the active invasion of tumour cells into surrounding brain tissue.[1] The invasiveness of GBM cells depends on various factors, such as tumour cell motility, cell-cell cohesion, cell-extracellular matrix (ECM) adhesion, and the structure of ECM.[2] The corticosteroid drug dexamethasone (DXM) is often used before surgery and during adjuvant therapy to alleviate cerebral oedema; however, the effects of DXM during anti-GBM treatment remain controversial.[3,4] Despite the growing interest in the effects of DXM in antiglioma therapy, there are no studies on its effects on the ECM of normal brain tissue and thus its influence on tumour invasion has not been comprehensively investigated.

In this study, we investigated the effects of DXM on brain ECM in *ex vivo* organotypic brain tissue culture and an *in vivo* experimental animal model using reverse transcription polymerase chain reaction (RT-PCR) and immunohistochemistry analyses. As the ECM of the brain tissue consists mainly of hyaluronic acid and complex protein-carbohydrate molecules, named proteoglycans (PG), the expression levels of the main PG in the normal brain tissue were determined before and after treatment with different DXM doses and regimens. According to RT-PCR analysis, syndecan-1, glypican-1, decorin, biglycan, and lumican were shown to be the most expressed PG in rat brain tissue in both *ex vivo* and *in vivo* models. In the *ex vivo* organotypic hippocampus culture, DXM treatment led to dose-dependent suppression of brevican, perlecan, and biglycan expression, and an increase in expression of glypican-1, neural/glial antigen 2, and versican. As different brain zones have specific expression patterns of PG, we analysed the effect of different doses and treatment regimens of DXM on PG in the rat cortex and hippocampus *in vivo*. Low-dose DXM treatment led to a significant decrease in the expression of most PG in the cortex but a 3-fold increase in syndecan-1, perlecan, and brevican expression in the hippocampus. Treatment with a high dose of DXM resulted in a 2–6-fold increase in the expression of most PG in both brain zones. Long-term treatment led to the most dramatic changes in PG expression on both messenger RNA and protein levels, completely changing their expression patterns.

Taken together, the data show that DXM treatment significantly affects PG expression in normal brain tissue. High doses of DXM and long-term treatment lead to the most dramatic alteration of PG composition in brain ECM. As extracellular PG play an important role in glioma cell proliferation and invasion, such alterations can create the appropriate microenvironmental niche for cancer cell

proliferation and tumour progression. In conclusion, the revealed effects of DXM on tumour-surrounding normal brain tissue suggest that DXM treatment might contribute to the negative side effects of the anti-glioma therapy, and the dose and treatment regimen are principally important.

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EXPERIENCE IN MANAGING PATIENTS WITH CARCINOID SYNDROME FROM A EUROPEAN NEUROENDOCRINE TUMOUR SOCIETY CENTRE OF EXCELLENCE

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Keywords: Carcinoid syndrome, neuroendocrine tumours, retrospective study, patient outcomes.

At the European Society of Medical Oncology (ESMO) 2017 congress, data from a tertiary referral centre (The Christie NHS Foundation Trust, Manchester, UK) were presented on the outcomes of 139 patients with carcinoid syndrome, identified from 882 patients treated for a gastroenteropancreatic neuroendocrine tumour (NET).

Carcinoid syndrome is a combination of signs and symptoms, such as skin flushing, diarrhoea, bronchospasm, and fibrotic valvulopathy, resulting from advanced NET secreting active mediators, such as serotonin and kallikrein.[1] Treating manifestations of the disease and preventing complications, in addition to achieving tumour control, can prove challenging. Due to the rarity of NET and carcinoid syndrome, there are few studies that present real-world data from large patient populations.

The prevalence of carcinoid syndrome reported in this presentation (16%) was similar to that indicated by Halperin et al.[2] (19%) in the 1st large epidemiological study, focussing on data from the American Surveillance, Epidemiology, and End Results (SEER) database linked to Medicare claims, in the field of NET. The frequencies of cardinal carcinoid symptoms in patients seen at The Christie NHS Foundation Trust were slightly higher than in previous reports,[3,4] but, similarly, flushing and

diarrhoea were by far the most common and were both present in 91% of patients, while carcinoid heart disease was described in 35% of patients.

Carcinoid syndrome is usually associated with liver metastases because of the hepatic clearance of active mediators released into the bloodstream by a primary tumour in the gastroenteropancreatic tract;[3] however, 18 patients (13%) in this study did not have liver metastases. Possible explanations for this are the influence of portacaval anastomoses; metastases in organs not drained by the portal circulation, such as lung (2 patients) and ovary metastases (1 patient); and the presence of occult liver metastases.

There was a positive association between the symptomatic and biochemical response to treatment, assessed through measurement of baseline and during treatment levels of 5-hydroxyindoleacetic acid (5-HIAA) in the serum or urine. Patients were divided into three groups correlating with symptom control: symptoms normalised on treatment, partial symptom control, and no symptomatic benefit. For all treatment lines, it was possible to ascertain that patients who achieved partial (p=0.049, p=0.001, respectively) and total symptom control (both p<0.001) for flushing and diarrhoea had a greater decline in 5-HIAA levels after treatment initiation than patients with no improvement in symptoms. However, changes in 5-HIAA levels 6 months after treatment initiation were not prognostic for progression-free (PFS) or overall survival (OS). Nevertheless, improvements in symptoms may positively impact quality of life; however, this element could not be assessed in this retrospective study.

Median follow-up was 45.7 months and revealed a median PFS of 27.0 months and OS of 65.4 months, similar to results reported by Halperin et al.[2] (OS: 60 months). Most patients (92%) were offered somatostatin analogues during their disease course, with high proportions undergoing liver embolisation (30%) or debulking surgery (23%). Surgery in the palliative setting is indicated in patients with carcinoid syndrome to aid symptom control and to prevent obstructive complications of a primary bowel tumour. Indeed, primary *in situ* was a negative prognostic factor for OS on multivariable analysis (hazard ratio [HR] 2.23; p=0.03). Other independent prognosticators were high expression of Ki-67 antigen (HR: 1.06; p=0.049) for worse PFS and baseline 5-HIAA levels for both PFS and OS (HR: 1.03; p=0.001 and HR: 1.02; p=0.04, respectively).

This is, to the best of our knowledge, one of the largest cohorts to date of real-world patients with gastroenteropancreatic NET and carcinoid syndrome from a single tertiary referral centre to have been analysed for clinical features and prognostic factors, and should inform future trial design in this rare subgroup.

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CANCER PATIENT ATTITUDES AND PREFERENCES TOWARD SMOKING STATUS ASSESSMENT

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Keywords: Smoking cessation, cancer survivorship, patient education.

Tobacco smoking affects the development, treatment response, prognosis, and recurrence of a variety of cancers.[1-4] In addition, it affects non-cancer cardio-respiratory outcomes.[1,3,5] In the general cancer patient population, about 50% of patients report a smoking history.[1] Although many patients quit smoking before they are diagnosed with cancer, about 20% of patients smoke within the year they are diagnosed with cancer.[1] Recent data by this study group has shown that up to half of cancer patients at our regional cancer centre who smoke in the peri-diagnosis period continue to smoke afterwards.[6]

Prior studies[3,4,7] found that most oncologists ask about smoking status only on the 1st assessment. Many cancer care providers report inadequate time and lack of skills to counsel effectively. Assessment and discussion of smoking status by physicians has been identified as a cause of discomfort and stigma among cancer patients who smoke.[8] Here, a cross-sectional survey of cancer patients was conducted across multiple disease sites and smoking statuses to assess the frequency of smoking assessment and the associated attitudes and preferences with respect to the screening of smoking status and discussion of smoking cessation.

It was identified that current smokers at diagnosis had their smoking status assessed more frequently than never or ex-smokers, but <50% of patients were being screened at ≥50% of their follow-up visits. Almost all patients (98%), regardless of smoking status, felt that it was important for their oncologist to be aware of their smoking status, and 95% wanted this information available at their 1st visit. Although current smokers were less likely to feel comfortable, compared to never or ex-smokers, with informing their oncologists of their smoking status at diagnosis (88% versus 98%, respectively; p<0.001), 96% were comfortable with being assessed. Although only half felt that smoking status should be assessed at all visits, among current smokers at diagnosis, lung cancer patients were 2–3 times more agreeable to being assessed at every visit, compared to patients with head and neck or non-tobacco related cancers. In general, patients felt that smoking status should be obtained by their oncologist (88%), as compared to other methods, including through allied health members, questionnaires, and electronic surveys (<50%); most patients (76%) felt that smoking cessation discussions should be initiated on the 1st visit.

Taken together, these survey results suggest that the vast majority of patients were comfortable with having their smoking status assessed, felt that this information was important, and were agreeable to having discussions initiated around tobacco cessation on the 1st visit by their oncologists, despite it being a potentially overwhelming time period. Currently, routine standardised screening, assessment, and treatment of tobacco use have not been consistently performed across all cancer centres.[9] The peri-diagnosis period during the initial cancer visits for a patient can provide a window of opportunity and offers a teachable moment to

discuss behavioural changes in patients, including tobacco cessation to optimise treatment response, reduce side effects, and improve prognosis. Pilot programmes, including one at our cancer centre, have shown that patients who are routinely screened are receptive to engaging in tobacco cessation programmes.[9,10] However, further methods to implement routine care pathways, which include systematic screening, strong recommendation to stop by the oncologist with access to medication, and counselling by other members of the team, are necessary for whole patient care. Counselling should include the harms of continued smoking and the benefits of stopping on side effects and treatment response. Future research on optimal treatment regimens and implementation strategies to make tobacco addiction treatment routine in cancer centres is warranted.

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RANDOM OPTIMISATION INTERACTIVE SYSTEM BASED ON KERNEL LEARNING (RISK) FOR VENOUS THROMBOEMBOLISM RISK ASSESSMENT IN CHEMOTHERAPY-TREATED CANCER PATIENTS

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Keywords: Venous thromboembolism (VTE), cancer, risk assessment, clinical decision support systems, machine learning, multiple kernel learning (MKL), random optimisation.

Venous thromboembolism (VTE) risk assessment is a major challenge in cancer outpatients treated

with chemotherapy, as its development may result in increased morbidity and treatment delays, with detrimental effects on the overall outcome and quality of life.[1] Although appropriate thromboprophylaxis could substantially improve patient care, it still remains sub-optimal and rather empiric, as highlighted at the European Society for Medical Oncology (ESMO) 2017 congress by Scotté et al.,[2] who stressed that the uncertainty regarding whether to use thromboprophylaxis reflects the absence of clear guidance.

Indeed, current guidelines do not recommend routine prophylaxis for VTE primary prevention in ambulatory cancer patients receiving chemotherapy, especially those under adjuvant and/or hormone treatment.[3,4] Nonetheless, as stated in the 2017 updated ESMO Clinical Practice Guidelines on supportive care, thromboprophylaxis 'may be considered in high-risk patients receiving palliative chemotherapy for locally advanced or metastatic disease.'[5] This statement emphasises how selecting patients for prophylactic anticoagulation is perceived as a growing necessity in cancer management, fostering the demand for risk assessment models (RAM).

Presently, pre-chemotherapy assessment of individual VTE risk is based on the Khorana Score (KS),[6] the sole validated RAM for cancer outpatients. However, even though KS is a user-friendly predictor based on routinely available variables, it strongly depends on tumour type and does not consider certain cancer types associated with VTE (e.g. brain tumours) or treatment-related factors. Therefore, its major limitation is that >50% of patients fall into an unclear group.[7,8] Accordingly, expanded RAM, including anti-cancer drugs or biomarkers, have been proposed, but a recent comparative analysis objected to the use of any of the available models.[9]

These considerations were the subject of discussion within the ESMO 2017 congress, where we presented a novel approach to developing VTE classifiers and a web service supporting the oncologists in risk assessment. The rationale was based on our recent demonstration that a machine learning approach based on multiple kernel learning (MKL), combining support vector machine algorithms and random optimisation, is capable of exploiting significant patterns in routinely collected demographic, clinical, and biochemical data.[10,11]

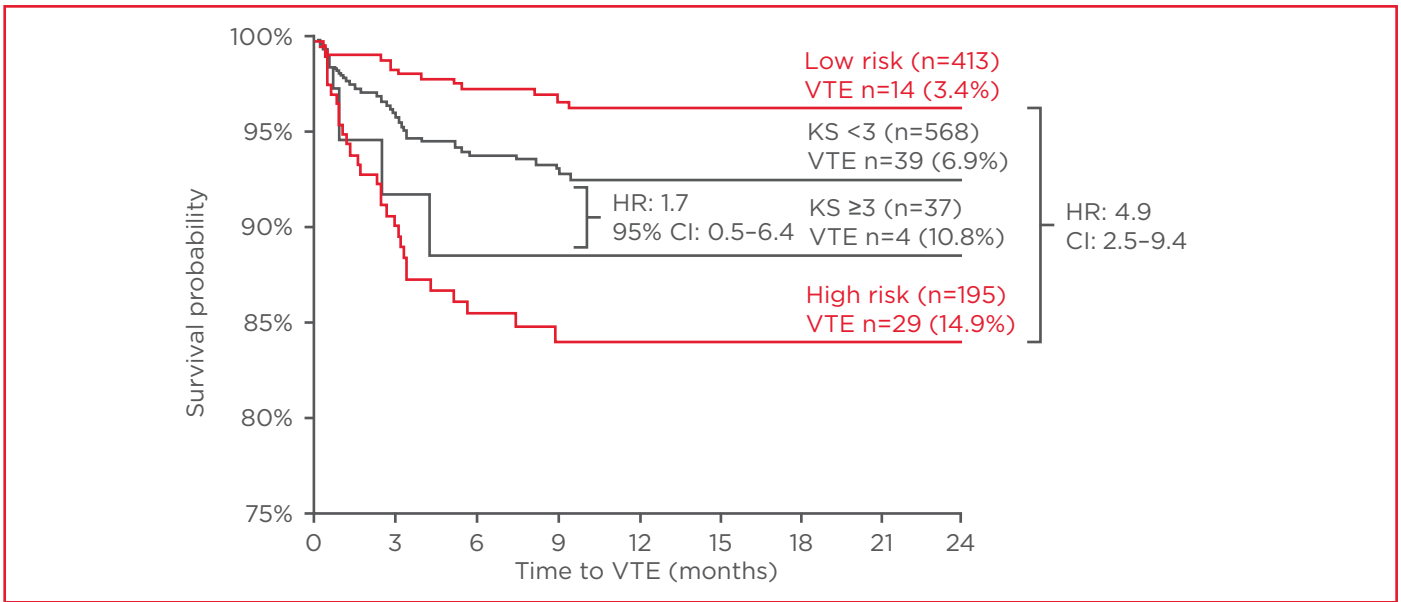

Figure 1: Kaplan-Meier curves of venous thromboembolism-free survival of chemotherapy-treated ambulatory cancer patients.

Comparison between patients stratified into low or high risk using the Khorana Score (black lines) or the VTE RISK score (red lines).

CI: confidence interval; HR: hazard ratio; KS: Khorana Score; VTE: venous thromboembolism.

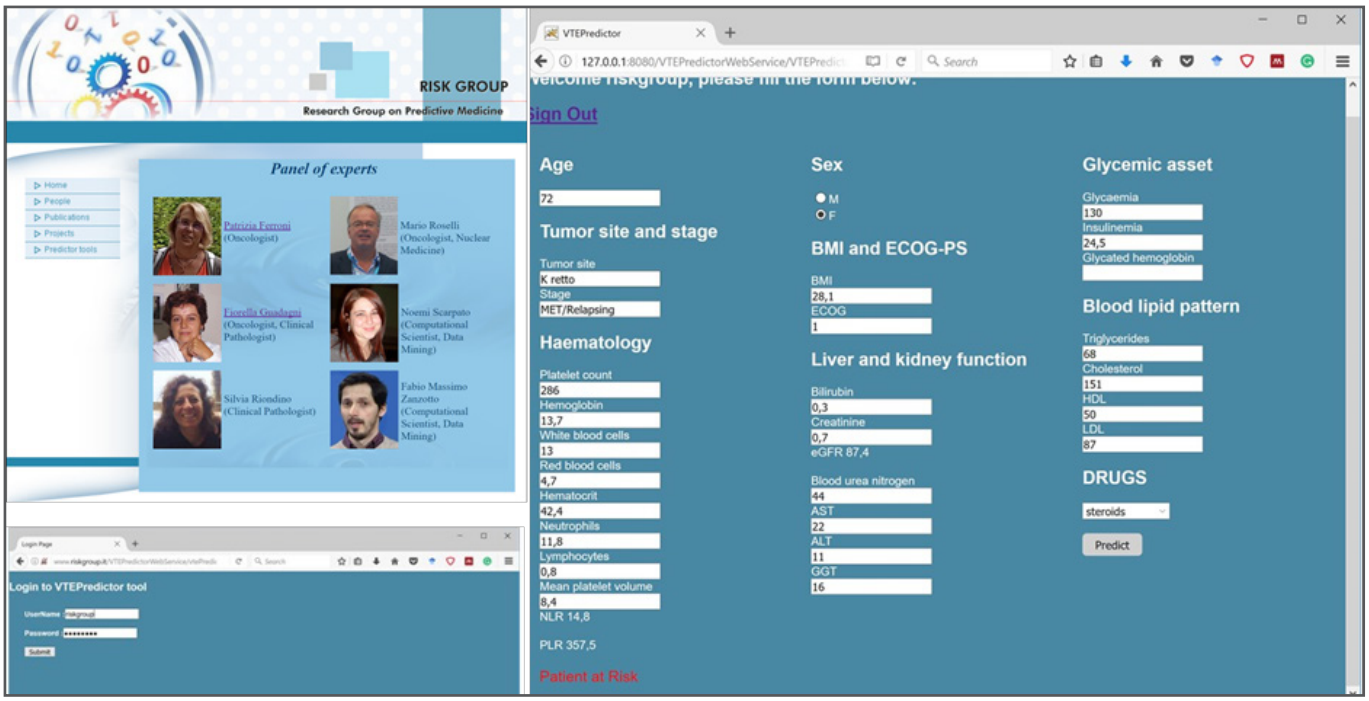

Figure 2: RISK Web Predictor.
Client form response[12] (www.riskgroup.it).

This results in a substantial advantage over traditional RAM (e.g. the KS, as reported in Figure 1), which are based on the arbitrary assignment of a score according to association analyses.

The VTE RISK Web Predictor is a web service with a graphical interface of risk model, based on a set of two MKL predictors (Figure 2). This service has two main components: a web interface (client) and a decision server. The web interface allows users to insert values for VTE risk evaluation and displays a prevision message. The interface is designed to help oncologists input data into the system. Hence, the client web form provides automatic suggestions and lists of options for the fields, and calculates values by using others provided by users. At the request of the user, the servlet contacts the decision server (i.e. the reasoning component that implements the kernel function), which computes the risk and returns estimates in the client form, even in the event of missing data, because the MKL allows some empty values in input form. This is represented by a binary value (0/1) achieved by both predictors according to a voting on the positive class.

In the context of developing predictive models, the use of MKL does not only provide an advantage over traditional models but, most importantly, retains several advantages in a perspective of precision medicine, with possible recalculation of updated data over time. The web interface will be publicly available shortly. We welcome the opportunity to invite interested researchers in its clinical application.

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SIGNAL TRANSDUCTION PATHWAYS REGULATING ALTERNATIVE SPLICING OF TUMOUR-RELATED RAC1B

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The expression of most genes in the human genome can yield >1 transcript through the process of alternative splicing of pre-messenger RNA. Alternatively spliced transcripts significantly increase the complexity of human gene products because they either influence transcript degradation levels or encode functional protein variants that differ in specific domains.[1] Even with cutting-edge transcriptomic approaches, it has been extremely challenging to understand or predict the complex splicing patterns observed in tissues or in diseases such as cancer; therefore, a better understanding of how cells are able to regulate alternative splicing is required.

Our group previously characterised the molecular mechanisms regulating RAC1b, an alternative splice variant that is overexpressed in colorectal tumours. RAC1b displays altered activation and downstream signalling properties and is required for colorectal cancer cell survival.[2] The method used by tumour cells to enhance this alternative splicing event remains largely unknown.

Alternative splicing is regulated through the binding of splicing factors to gene-specific sequence elements and can be modified by changing either the expression levels of competing factors, or their activity and subcellular localisation, through protein phosphorylation.[3] The study of RAC1b revealed that two antagonistic SR protein-family splicing factors, SRSF1 and SRSF3, bind to regulatory sequence elements in the alternative exon and determine the rate of exon inclusion. While the inhibitory factor SRSF3 was found to be regulated at the

transcriptional level, the antagonistic SRSF1 was regulated through protein phosphorylation.[4] Interestingly, recent data revealed that colon epithelial cells increase RAC1b expression in an inflammatory environment, indicating the role of cytokine signalling as an upstream event that is necessary for changes in alternative splicing.[5] Here, we explore how signalling pathways are involved in the deregulation of alternative RAC1b splicing in colorectal tumour cells.

Using HT29 cells that represent serrated colorectal tumours, with both the *BRAF* gene mutation Val600Glu in one allele and RAC1b overexpression, we depleted 20 candidate splicing-regulatory protein kinase genes by RNA interference. It was found that AKT2, AKT3, GSK3β, and SRPK1 are all required to sustain RAC1b levels. While knockdown of AKT2 and AKT3 affected only RAC1b protein levels, suggesting a post-splicing effect, the depletion of GSK3β or SRPK1 decreased RAC1b alternative splicing, an effect mediated through changes in splicing factor SRSF1. In particular, the knockdown of SRPK1, or pharmacological inhibition of its catalytic activity, reduced phosphorylation and subsequent translocation of SRSF1 to the nucleus, limiting its availability to promote the inclusion of alternative exon 3b into the RAC1b pre-messenger RNA.[6] This regulatory pathway was also found to be controlled by GSK3β. Interestingly, GSK3β phosphorylation was identified as a target of the anti-inflammatory drug ibuprofen, which inhibits RAC1b overexpression.

This work advances the current research on aberrant splicing events in cancer and demonstrates that oncogenic signal transduction pathways deregulate alternative splicing. Moreover, our results show that alternative splicing may be drug revertible and promote exploitation of the growing list of kinase inhibitors as a therapeutic resource to correct splicing in cancer cells.

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IDEAL CARDIOVASCULAR HEALTH IN PATIENTS WITH A RECENT DIAGNOSIS OF COLORECTAL CANCER

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Keywords: Ideal cardiovascular (CV) health, cancer survivors, cardiovascular (CV) risk factors, colorectal cancer, physical activity, healthy lifestyle.

Colorectal cancer survivors have an elevated risk of comorbid disease, particularly cardiovascular (CV) disease, due to both the age at diagnosis (around 60 years) and shared lifestyle risk factors; namely, being overweight/obese, physical inactivity, poor diet, and smoking.[1,2] BMI is the strongest correlate of comorbid CV disease in cancer survivors.[2] Comorbid chronic conditions can have

a negative impact on colorectal cancer survival and can be a cause of death in these patients.[3] In fact, coronary artery disease is the leading cause of death in colorectal cancer survivors 10 years after diagnosis. There is mechanistic evidence that obesity induces alterations in proinflammatory cytokines, lipid metabolites, adipokines, and insulin growth factor signalling pathways.[4]

Health behaviours seem to have an impact on outcomes after the diagnosis of colorectal cancer.[5] There is strong evidence of the benefits of physical activity in cancer outcomes after the diagnosis of colorectal cancer.[6] Unfortunately, most of these studies were conducted using a questionnaire, which is very well-known to constitute an important bias, since patients overestimate physical activity and underestimate sedentarism.[7] A number of studies have documented the prevalence of comorbid chronic conditions in colorectal cancer survivors.[3] However, there is no study of cancer survivors that focusses on the current American Heart Association (AHA) policy on CV health. To define CV health, the Committee of the Strategic Planning Task Force of the AHA adopted positive language, defining health factors instead of risk factors, and health behaviours instead of risk behaviours.[8] Whereas behaviours are modifiable, factors are not.

The objective of our study was to describe the CV health of a cohort of recently diagnosed colorectal cancer patients. The criteria used to define an ideal CV health in our sample were very restrictive. In order to adhere to current recommendations, ≥150 minutes of moderate-to-vigorous physical activity needed to be recorded through accelerometers. Diet was evaluated using the PREDIMED questionnaire, which evaluated the adherence of participants to a Mediterranean diet. Patients who did not report CV risk factors had their values for blood pressure, BMI, glucose, and cholesterol measured at hospital (Table 1). Ideal CV health was considered only when both four behaviours and three health factors were present at the same time.

Of the 91 patients who were recruited, only 1 patient achieved seven metrics, which represented 1.1% of our sample having ideal CV health. Even when objectively measured, our sample was overall compliant with physical activity recommendations, confirming previous findings in Spanish cancer survivors.[9] Being overweight/obese was the most prevalent unhealthy behaviour.

Becoming overweight or obese is the result of chronic energy imbalance. A significant controversy in the field is the so-called 'obesity paradox', which confers a better prognosis to obese metastatic colorectal cancer survivors.[10] The relationships between energy balance and prognosis, sarcopenia, sarcopenic obesity, and cancer outcomes, in addition to the biologic mechanisms that mediate this relationship, are promising areas that warrant additional investigation.

Table 1: The cardiovascular health of a cohort of patients with recently diagnosed colorectal cancer.

91 colorectal cancer survivors (36% metastatic); median age 65 years; 69% male; 31% female.								
ICVH	No CV disease history	Health behaviours				Health factors		
		Non-smoking	BMI <25 kg/m^2	Healthy diet (PREDIMED) >8/14	MVPA 150 minutes/ week	No HT (<120/<80 mmHg)	No DM (<100 mg/dL fasting plasma glucose)	No DL (<200 mg/dL total cholesterol)
1.1%	91%	90.5%	36%	67%	96%	51%	84.3%	66%

CV: cardiovascular; DL: dyslipidaemia; DM: diabetes mellitus; HT: hypertension; ICVH: ideal cardiovascular health; MVPA: moderate-to-vigorous physical activity.

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DETERMINING THE ROLE OF ETS FACTOR ELF3/ESE-1 IN THE NORMAL AND MALIGNANT PROSTATE

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Keywords: *ELF3* gene, ETS factors, prostate cancer.

Prostate epithelial cells exist as a differentiation hierarchy, which is structured as two layers. The basal layer is the more proliferative compartment of the epithelium and is comprised of three different cell types: stem cells, transit amplifying cells, and committed basal (CB) cells. CB cells differentiate to form the luminal layer of the epithelium, which consists of terminally differentiated, secretory luminal cells. In prostate cancer, epithelial differentiation is deregulated, resulting in the growth and expansion of the luminal population. Treatment strategies for advanced prostate cancer currently focus on androgen ablation therapies, which target the androgen-sensitive luminal population. However, evidence suggests that prostate cancer arises from the basal population, which is not targeted by current treatments.

In prostate cancer, the most prevalent genetic abnormality is the *TMPRSS2:ERG* fusion. *ERG* is a member of the ETS factor family, members of which are widely known to be involved in several types of cancers, including leukaemias. The aim of this study was to define the role of the epithelial-specific ETS transcription factor ELF3 in normal prostate epithelium, as well as its role in prostate cancer. ELF3 has been described as both a driver and repressor of cancer in different tissues, including prostate cancer. Similar findings for other ETS factors suggest that ELF3 may function in a highly cell type-dependent, and context-dependent, manner.[1]

A gene expression microarray, with RNA derived from benign and cancerous prostate tissue, indicated that ELF3 was significantly more highly expressed in the CB cell fraction compared to stem cells, regardless of pathological diagnosis. At the protein level, consistently higher ELF3 expression

was seen in the CB cell population compared to the less differentiated transit amplifying cells, and ELF3 was undetectable in luminal epithelial cells or stroma, by both immunohistochemistry and western blot.

BPH-1 (benign) and PC3 (cancer) cell lines were chosen as suitable ELF3-expressing basal cell models to investigate the effects of knockdown of *ELF3*. Using small interfering-RNA transfection, *ELF3* knockdown reduced the migration, clonogenicity, and viability of prostate epithelial cells over time, regardless of origin. A gene expression microarray performed on BPH-1 and PC3 cells after *ELF3* knockdown, revealed significant changes related to control of the cell cycle, which was consistent with the functional changes seen. Changes in cell cycle protein expression matched the RNA expression results. Preliminary experiments suggest *ELF3* knockdown causes an arrest at the G2 phase, which equally correlates with the specific gene changes seen in the microarray.

Current work, including *ELF3* overexpression and *ELF3* knockdown in primary prostate epithelial cultures derived from patient tumour biopsies, will provide clinically relevant evidence as to the precise role of *ELF3* in the prostate. Further experiments are also being carried out using quantitative phase imaging to analyse the rapid, real-time changes in cells after *ELF3* expression manipulation. What emerges from these studies is that rather than acting as a facilitator of tumour progression, *ELF3* appears to play a prominent role in prostate epithelial cell differentiation.

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THE ROLE OF PSYCHOLOGICAL STRESS AND NITRIC OXIDE SYNTHASE INHIBITION IN BREAST CANCER

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Keywords: Breast cancer, stress, DNA damage.

Psychological stress has been implicated as a risk factor in the progression of breast cancer, however the biological mechanisms are not well understood. In animal models of psychological stress, an increase in mammary tumour burden and metastatic spread has been observed.[1] Furthermore, in breast cancer cells, the stress hormones cortisol and noradrenaline, released as part of the stress response, have been shown to promote DNA damage through the generation of reactive oxygen/nitrogen species.[2]

The research presented here aimed to first explore the *in vitro* effects of cortisol by measuring the induction of DNA damage and the generation of nitric oxide (NO). An *in vivo* mouse model of psychological stress was also employed to study the impact of stress on the progression of breast cancer, alongside an inhibitor of nitric oxide synthase (NOS) (L-NAME). Through pharmacological inhibition of the glucocorticoid receptor (GR), as well as selective and non-selective inhibition of NOS, this research aimed to elucidate a mechanism through which stress may impact breast cancer progression.

In mammary tumour cells treated with cortisol, an increase in both NO and DNA damage was observed. This was abrogated with inhibition of the GR, as well as pan-inhibition of NOS and, specifically, the isoform inducible NOS (iNOS).

Cortisol upregulated the expression of iNOS, a marker previously suggested as an indicator of poor survival in breast cancer.[3] Induction of chronic stress in a syngeneic mouse model of breast cancer had no effect on the growth of the primary tumour; however, inhibition of NOS in stressed mice significantly decreased tumour volume compared to stress alone. A significant increase in tumour microvasculature was observed in the primary tumours, as there was an increase in metastatic sites per mouse in stressed mice, compared to the control. This was also reversed through the inhibition of NOS.

This study indicated that stress may impact tumourigenic progression through the induction of DNA damage, mediated by the release of NO. Inhibition of NOS was able to negate the effects of stress on tumour growth and metastasis, providing an insight into the potential benefits of NOS inhibitors in breast cancer treatment. However, presentation of this research at the ESMO congress raised some potential implications of inhibition of NO signalling. Primarily, these included the effects of NOS inhibition on haemodynamics, because L-NAME has been used previously in a clinical setting to increase blood pressure. In future studies, this limitation could be resolved through cotreatment with antihypertensives, as was the case in a previous study that demonstrated the growth inhibitory effects of iNOS inhibition in breast cancer.[4]

Discussion also focussed on the effects of glucocorticoid signalling in the immune system, since previous research has demonstrated that stress can adversely affect the immune response in breast cancer models, and in patients.[5,6] Furthermore, an increase in the expression of the GR has been observed to correlate with poor prognosis in oestrogen receptor-negative breast cancer patients, indicating that glucocorticoid-mediated signalling can influence cancer progression.[7] As such, current trials using novel GR inhibitors in breast cancer patients are underway. Future perspectives for this work would seek to use pharmacological therapeutics, including using selective GR inhibitors in combination with NOS inhibitors, in highly stressed patients with breast cancer.

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REGULATION OF GLUCOSE TRANSPORTERS BY PROTEIN KINASES IN CANCER CELLS

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Keywords: Cancer cell, glucose transport, signalling pathways, WNK protein kinase.

Reprogramming of energy metabolism is one of the hallmarks of cancer, since tumour cells need to fuel their high proliferation rates.[1] Warburg et al.[2] first observed that cancer cells largely limit their method of energy production to glycolysis only. In order to compensate for lower ATP yields, cancer cells often upregulate expression of glucose transporters, typically GLUT1, which substantially increases extracellular glucose import into the cytoplasm.[3]

In normal physiology, insulin-responsive cells rapidly increase their glucose uptake 10–20-fold by translocating glucose transporter vesicles (specifically GLUT4) from intracellular storage compartments to the plasma membrane. In adipocytes, insulin activates the protein kinase AKT to phosphorylate the Rab GTPase activating protein, TBC1D4. This protein normally maintains Rab GTPases in their inactive state so that storage vesicles containing the glucose transporter remain cytoplasmic. Upon phosphorylation, TBC1D4 becomes inactive, allowing the translocation of GLUT vesicles so that more transporters are inserted into the plasma membrane.[4] In muscle cells, the highly homologous Rab-GTPase activating protein TBC1D1 is predominantly expressed but also becomes phosphorylated by AKT in response to insulin.

Previous work from our laboratory described that the protein kinase WNK1 also participates in the regulation of TBC1D4. WNK1 forms a protein complex with TBC1D4 in human embryonic kidney (HEK293) cells and phosphorylates it *in vitro*.

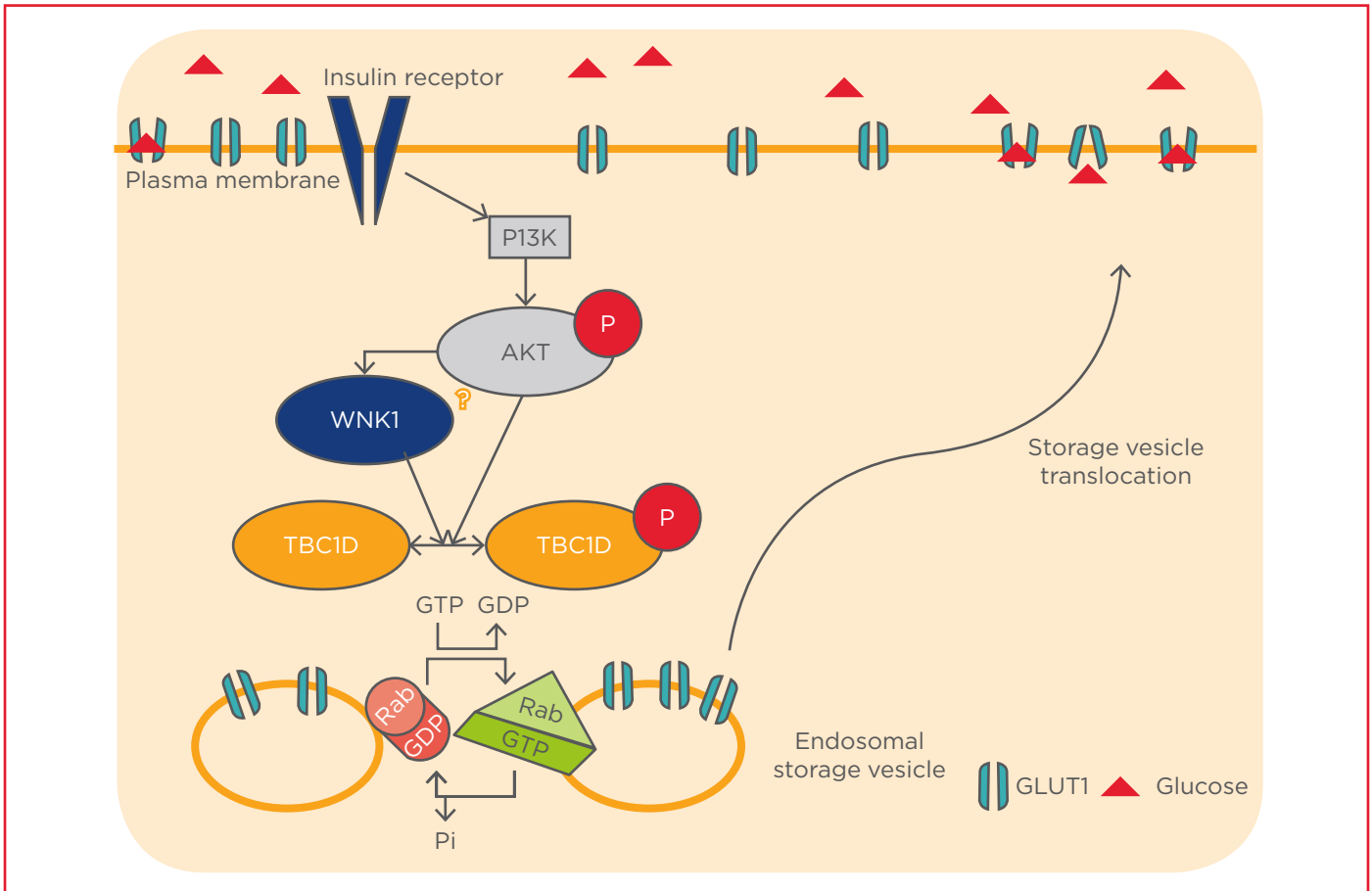

Figure 1: The regulation of cell surface expression of GLUT1.
GLUT1: glucose transporter 1; PI3K: phosphoinositide 3-kinase.

Phosphorylation by WNK1 was found to increase the binding of TBC1D4 to regulatory 14-3-3 proteins and to reduce its interaction with the exocytic small GTPase, Rab8A. This novel pathway regulates the cell surface expression of GLUT1 (Figure 1).[5]

The subfamily of WNK protein kinases belongs to the superfamily of 518 human protein kinase genes.[6,7] WNK1 and WNK4 were shown to regulate the cell surface expression and activity of a variety of renal ion channels.[7] The finding that WNK1 regulated GLUT1 showed, for the first time, that it is also involved in the cell surface expression of other plasma membrane transport proteins.[5] Besides the catalytic domain, WNK1 contains coiled-coil domains, which, together with the large size of the protein, suggests that WNK1 also has scaffolding functions and may be involved in multiple cellular signalling processes.[6-8] Interestingly, AKT can phosphorylate WNK1 at residue Thr60.[9]

Our goal was to characterise the role of protein kinase WNK1 in the phosphorylation network that regulates cellular glucose uptake in cancer cells. WNK1 expression was depleted by small interfering RNA in various colorectal cancer cell lines and key cell cycle proteins were analysed by a western blot in the presence of different glucose concentrations. WNK1-depleted cells showed higher apoptotic and cell cycle arrest phenotypes when cultured in low glucose medium.

In order to determine how WNK1 regulates GLUT1 translocation, key phosphorylation events were identified by mass spectrometry analysis, which revealed unique serine residues specifically phosphorylated by WNK1 in both TBC1D4 and the functionally related TBC1D1. The respective phosphomimetic mutants are currently being tested for their ability to modulate GLUT1 translocation. Together, these studies will contribute to an increased knowledge of the signalling pathways involved in the control of glucose uptake in cancer cells and will potentially allow the identification of novel therapeutic targets.

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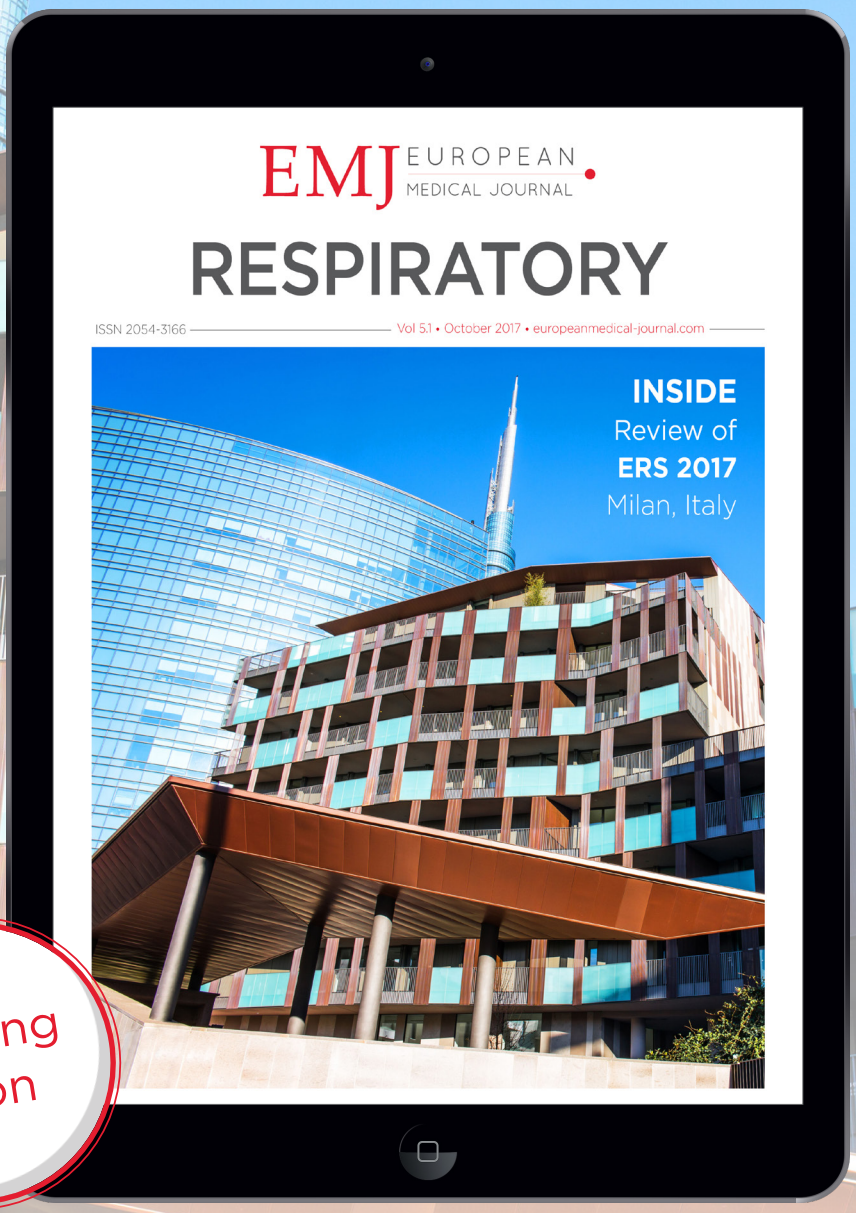

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EDITOR'S PICK

This issue's Editor's Pick is a relevant discussion paper from Lechuga et al. on the potential of simple, low-cost, and timely optical biosensors for the detection of epigenetic biomarkers in cancer diagnosis. The development of new methods for the detection and monitoring of cancer has been the subject of intense research over the last few years and the role of biosensors in the analysis of epigenetic biomarkers offers a promising solution.

Samantha Warne

SIMPLE, LOW-COST, AND TIMELY OPTICAL BIOSENSORS FOR THE DETECTION OF EPIGENETIC BIOMARKERS: THE FUTURE OF CANCER DIAGNOSIS

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ABSTRACT

The cancer burden continues to grow with enormous physical, emotional, and financial pressure on individuals, families, communities, and health systems. Early detection and effective treatment are crucial. The analysis of epigenetic biomarkers is presented as an exceptional solution for early cancer diagnosis and personalised treatment design. These brand new biomarkers have initiated a diagnostic revolution because of their predictive capability and reversibility, opening the window for timely diagnostics and personalised medicine. In recent years, the potential of optical biosensors for epigenetic biomarker evaluation has been revealed. Nanotechnology is promoting the appearance of new advanced biosensors able to be integrated in complete lab-on-chip platforms. Lab-on-chip biosensors are offering simplified, cost-effective, and fast results to solve the current diagnostic problems. In this review, we present the advantages offered by the analysis of epigenetic routes in cancer diagnosis and the current advances in optical biosensors for cancer epigenetic analysis, showing how the new biosensor solutions manage to surpass the challenges encountered during the analysis of each epigenetic mechanism.

Keywords: Cancer diagnosis, biosensors, nanomedicine, biomarkers, biotechnology, epigenetics, gene regulation pathways.

INTRODUCTION

Cancer is one of the leading life-threatening diseases across the world, with 200 different types of cancer accounting for >1,500 deaths per day.[1] The main constraints in cancer management can be found in two principal problems: late diagnosis and low efficiency of therapies. Despite the recent technological advancements, cancer diagnosis is still an unfinished issue for physicians, since many cancers remain asymptomatic until relatively late stages, leading to a poor survival rate.[1] The continuous failure of many treatments against late-diagnosed cancers is forcing scientists to establish new formulas for cancer prediction and staging. A better definition of the tumour origin and its stage of development will reduce the possibility of treatment defeat. Current conventional methods are inefficient for early stage cancer detection, as they are based on the evaluation of the phenotypic properties of the tumour.[1] In addition, these techniques are invasive, expensive, and rely on time-consuming procedures carried out in specialised laboratories. Regarding the applied therapies, most of them are not as precise and accurate as required, resulting in low success rates or devastating side-effects, which makes the identification of more specific drugs an indispensable requisite, and proper doses that best benefit each individual.

There are several molecules that express prominent alterations in their expression during cancer, recognised as biomarkers and having high clinical significance. The presence, absence, or change in the level of these specific biomarkers in a cell, tissue, or biofluid often indicates cancer development.[2,3] Cancer is a multistage disease of which onset and progression are associated with a complex variety of genetic or epigenetic alterations, resulting in tumourigenic transformation and progression.[4] Epigenetics literally means 'above' or 'on top of' genetics. It refers to any heritable trait that is not derived from the DNA sequence. These inherited characteristics are transmitted to offspring in the form of subtle chemical modifications to DNA and DNA-associated proteins, and exert their effects by modulating the gene expression. Epigenetic mechanisms are implicated in several processes, playing important roles in cellular decision-making and providing the cells with a powerful capability to readily change their genomic expression in order to survive and reproduce successfully in ever-changing environments. For that reason, the field of epigenetics is considered a promising solution for cancer prediction and eradication. New discoveries in the epigenetic regulation of cells are gaining more and more attention in diagnosis. These epigenetic mechanisms have been shown to play important roles during cell development and equilibrium maintenance. Chatterjee and Zetters'[2] study helped elucidate the shortcuts that cancer cells take by shifting these regulating routes for longer survival and proliferation over normal cells. Furthermore, several epigenetic changes occur prior to histopathological changes, constituting outstanding biomarkers for early cancer identification, diagnosis, and risk assessment.[3] With a better knowledge of epigenetic mechanisms, one would be able to understand when, why, and how a tumour is produced. In addition, the specific reversion of these routes represents an excellent therapy solution in order to return the cancer cells to their normal state, without implying invasive chemotherapies and post-treatment side effects.

Currently, new diagnostic devices are being developed in order to decipher and keep track of these epigenetic mechanisms.[4] These tools use highly advanced technologies that allow a simpler and more cost-effective diagnosis. The ultimate goal is to use the devices outside the laboratory for the continuous monitoring of the patient before and after treatment. Such devices may allow the simultaneous detection of multiple biomarkers belonging to a previously defined cancer epigenetic profile. In this context, nanotechnology is playing a decisive role by offering ultrasensitive devices with powerful and highly improved performances as compared to the current diagnostic techniques.[5] The combination of nanotechnology and epigenetics is advancing towards a new generation of cancer diagnostic tools, where the primary objective is to provide low-cost, simple, and ultra-detailed analyses of the patients' clinical conditions in record time. This early diagnosis will define a newer and hopeful starting point for cancer treatment, helping design specific protocols and targets for therapies specifically tailored for each individual.

This manuscript reviews the benefits provided by the study of epigenetic mechanisms and their relationship with cancer. We also present some emergent, brand new nanobiosensor technologies, aiming to provide an advanced diagnostic tool by analysing the epigenetic mechanisms altered in cancer. The new nanosensors accomplished

the premises of user-friendly, reduced costs, and improved performance, priming them to spark a diagnostic revolution.

THE CANCER EPIGENETIC SIGNATURE

Different epigenetic mechanisms control the gene expression at different levels, constituting a complex, highly structured gene regulatory network. One of the most relevant epigenetic processes is DNA methylation. It consists of the addition of methyl groups to cytosine nucleotides (5-methylcytosine [5-mC]) in the DNA sequence to impede transcription, aiding cells to modulate the expression of the implicated genes at their convenience.[5] In the same way, the cell can reorganise its chromatin structure with another epigenetic mark, the acetylation/deacetylation of its histones, affecting the transcription process. This epigenetic regulation of the transcription process has a strong influence at the post-transcription level, as in the case of the regulation of alternative splicing of messenger RNA (mRNA), which gives the cell the capability to alternatively edit a pre-mRNA to produce proteins with different functions, increasing diversity.[6] On the other hand, cells can regulate the gene expression through micro-RNA. These small, single-stranded, non-coding RNA typically contain 19–23 nucleotides and play important roles in modulating several biological functions through their interaction with mRNA.[7] In this way, cells will either translate or not a specific gene via the regulation of epigenetic control.

All these mechanisms are interconnected and play important roles in every process of life, including cell differentiation, metabolism, cell cycle, and signal transduction. Therefore, it is not surprising that their alteration disturbs the fate of cells and the consequences can be devastating, resulting in the origin of grave diseases, one of the most predominant of which is cancer. In fact, aberrant DNA methylation has been linked to the silencing of tumour suppressor genes, leading to the cancer development.[8] Most cancers are associated with a switch in the splicing pattern of specific isoforms, providing them with a highly proliferative capacity and survival properties.[9] Likewise, an aberrant expression of specific micro-RNA sequences has been directly linked to the generation of tumours.[10] As a consequence, these epigenetic mechanisms are now being considered hallmarks of cancer. Current cancer biomarkers are based on overexpressed cancer proteins in blood; however, their number and clinical use are rather limited and require a large population of cancer patients with well-defined clinical staging and outcomes. Analysis of the epigenetic pathways may be more informative, specific, and accurate than the analysis of such protein biomarkers, by the determination of not only the cancer itself, but also the underlying mechanisms by which it is generated. Recent studies indicate that these regulation pathways participate in collaborative activities resulting in a common outcome.[11] Understanding the dynamics of these networks can shed light on the mechanisms responsible for the development of many types of cancer. On top of this, it will contribute to a more efficient management of cancer patients, providing an early diagnosis, determining precise tumour staging, and monitoring of treatment.

The revolutionary epigenetic inheritance has changed how we understand and deal with cancer. The dynamic nature and potential reversibility of the epigenetic mechanisms mean that they are appealing therapeutic targets in cancer treatment. Applying therapies focussed on reversion of the altered processes to their normal state would abate the cancer progression in a less invasive and more efficient manner than standard chemotherapies. Currently, various compounds that can rearrange DNA methylation and histone acetylation patterns are being examined in clinical settings in combination with other drugs.[12,13] Likewise, different approaches for mRNA and non-coding-RNA expression control are currently being assessed in order to potentially use them in more directed therapies.[14-17] Therefore, a better knowledge of cell decision-making in cancer can be better exploited for the development and implementation of a more personalised medicine.

BIOSENSORS: THE DIAGNOSTIC SOLUTION

Advances in DNA/RNA detection techniques have recently hit the epigenetic field. Developments in real-time polymerase chain reaction (PCR) have allowed results to be obtained in a matter of a few minutes regarding the methylation status or mRNA isoform shift.[18,19] DNA microarrays permit the interrogation of thousands of micro-RNA sequences simultaneously in one sample,[20] while next-generation DNA sequencing technologies have expanded to genome-wide scale screening

and have achieved a resolution to single base precision.[21] However, these methods suffer technological limitations, such as slow turnaround times,[22] relatively large input volume,[23] and biases arising from sample contamination and PCR-induced artifacts.[24,25] They usually require the consumption of expensive reagents in every assay and need trained personnel for their manipulation due to the complexity of the analytical protocols. Besides, all cancers involve more than one epigenetic mechanism, making the simultaneous detection of multiple epigenetic biomarkers essential.

Emerging trends in diagnostics have promoted the development of diagnostic tools with improved sensitivity and short operation times, such as biosensors. Biosensors can be designed to provide quantitative analytical information with elevated accuracy in a few minutes, using low sample volumes and minimum sample pretreatment. By definition, a biosensor is a self-contained analytical device that incorporates a biologically active material in intimate contact with an appropriate transduction element for the purpose of detecting, in a very selective way, the concentration or activity of chemical species in any type of sample (Figure 1).[26]

Biosensors are threatening to radically alter our present concept of clinical analysis, beginning many years ago[27] with the introduction of the glucose biosensor signifying a breakthrough in healthcare by the decentralisation and simplicity of analysis at home by one blood-drop.[28] The principal aim of biosensors is to get away from the centralised laboratory and to provide analytical services closer to the patient: at the bedside, in the physician's office, or by the patient at home.

Nowadays, there are some commercial biosensors for several applications, such as detection of clinical biomarkers or pathogens and toxic metabolites for environmental/food contamination and bio-threats.[29] Biosensor devices can show extremely low detection limits, which permits the detection of biomarkers at their physiological concentrations, assisting in the diagnosis of cancer at very early stages. In the last decades, a particular interest has been focussed on the development of novel, label-free optical biosensors able to generate a signal directly by the interaction of the analyte of interest with the recognition element, without requiring additional interactions with other probes carrying a label that provides the signal. In general, label-free methods offer potential advantages in terms of simplicity and velocity of the bioanalysis, which may not require washing steps or additional reagents. These biosensors enable the real-time monitoring of the biomolecular interaction, speeding up detection and giving access to the kinetic parameters of the recognition process.[30] Moreover, with the advent of micro and nanotechnology, more sophisticated label-free, optical biosensors combine extremely high-quality performances and ultrasensitive limits of detection with the ability to miniaturise and integrate different functional components such as microfluidics, electronics, etc. in a single platform. These characteristics allow for the fabrication of smaller, cheaper, and easy-to-use biosensor devices that can accelerate the real implementation of lab-on-a-chip devices in clinical practice.

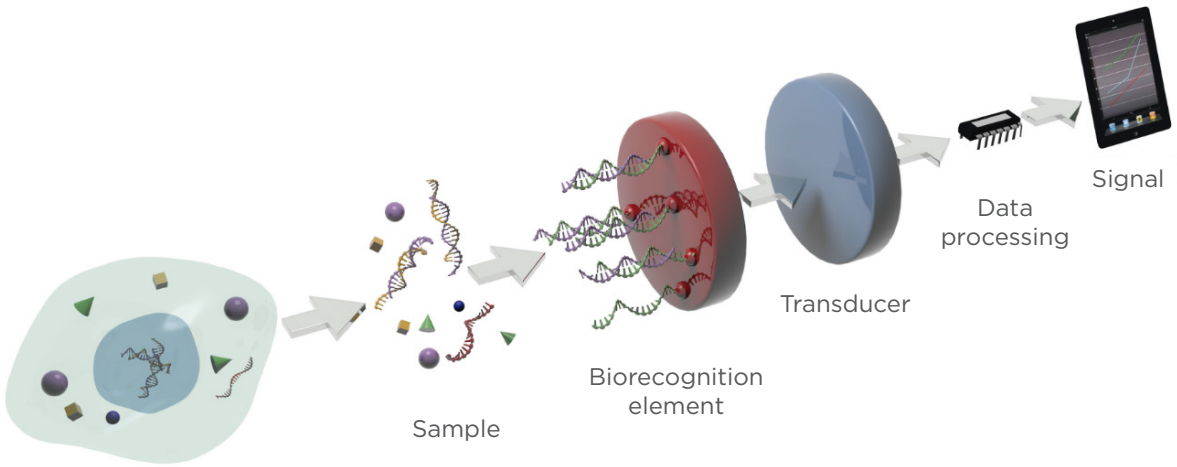

Figure 1: Schematic diagram of a biosensor device.
Adapted with permission from Carrascosa et al.[31]

Table 1: Challenges in the biosensing of epigenetic mechanisms and different biosensor approaches.

Epigenetic pathway	Detection drawbacks in conventional methods	Biosensor approaches
DNA methylation	False positive results due to errors in: • PCR amplification • DNA extraction • Bisulfite conversion Low sample input	Bisulfate conversion-based SPR biosensors[34,35] Antibody-based methyl recognition SPR biosensor[37,38] Methyl-binding protein-based SPR biosensor[39]
Micro-RNA	Small fraction of total RNA (~0.01%) Difficult to amplify Wide dynamic range: expression levels vary from a few copies to >50,000 copies per cell Sequence similarity among micro-RNA family members	Immuno-amplification-based SPR biosensor[40] Nanoparticle-amplification-based SPR biosensor[41,42] Triplex-forming probe-based SPR biosensor[43] Multiplexed immuno-amplification microring resonator-based biosensor[44] Multiplex nanophotonic interferometer biosensor[45]
Alternative splicing	Target accessibility (long sequences ≤1,000,000 nucleotides) Cross-hybridisation due to sequence similarities Low concentrations	Quantitative-imaging-based biosensor[46] SPR-based biosensor[47] Multiplex nanophotonic interferometer biosensor[48]

PCR: polymerase chain reaction; SPR: surface plasmon resonance.

Optical biosensors have shown exceptional capabilities for the detection of DNA/RNA sequences, at detection limits more than adequate for the physiological concentration of the target sequences in real samples.[31] All optical biosensors developed so far for epigenetic biomarkers are characterised by the commitment to overcome the challenges encountered in their analysis (see Table 1 for a summary).

For instance, the modification of the DNA sequence by methyl-groups has been of interest in many optical biosensor applications.[32] Some methodologies carried out analysis of DNA methylation through bisulfite conversion, employing a surface plasmon resonance (SPR) biosensor. This type of biosensor uses a gold layer as both a surface to immobilise specific probes and a transducer of the signals produced by the refractive index changes at the surface.[33] Bisulfite converts non-methylated cytosine nucleotides into uracil nucleotides, allowing the identification of different methylated or non-methylated sequences by different amplification processes, such as PCR[34] or molecular inversion probe amplification.[35] However, bisulfite conversion does not differentiate between other epigenetic marks derived from enzymatic oxidisation of 5-mC, with important significance in the determination of the methylation status.[36] Also, the possibility of bias introduced by errors in this pre-manipulation process has promoted the search for other alternatives. Specific recognition of methyl-sites (and derivatives) has been solved with specific antibodies[37,38] or proteins[39] being able to quantify the number of cytosines without the necessity of sample manipulation. In this way, new microfluidic designs have been proposed in order to provide smaller and more easy-to-handle equipment for DNA methylation analyses.[38] The microfluidics incorporate different pre-treatment processes on the same microchip as the methylation analysis, such as fragmentation by restriction enzyme to obtain the fragment of interest, mixture with a biotinylated bulge-inducer probe to improve the methyl-group accessibility, and heat denaturation and cool down for hybridisation.

In the case of micro-RNA detection, due to their small size, short RNA regulators are difficult to amplify through conventional methods. In addition, they usually belong to a micro-RNA family with very similar sequences that can distort the analysis with false positive signals. The main objective towards micro-RNA detection relies on two premises: specificity and a high sensitivity to cover a wide range of concentrations. The ability to perform the experiments directly from an untreated biofluid without the need for purification steps, thereby risking sample input, is also important.

Some SPR biosensors have been developed for the analysis of micro-RNA. Šípová et al.[40] demonstrated the detection of specific micro-RNA in mouse liver tissues. To achieve the required sensitivity levels without PCR-amplification steps, researchers used a signal enhancer based on a specific antibody.[40] Other signal enhancers have also been proposed, such as nanoparticles[41,42] or specially designed probes, which promote a better target capture.[43] Another approach based on microring resonator biosensors has been proposed.[44] The miniature size of the microrings offers the possibility of an array of biosensors (32 microring resonators within a 6×6 mm footprint) for multiplexed measurements, which is very encouraging for their use in routine clinics. On the other hand, our team has designed a nanotechnology-based optical biosensor with multiplexing capabilities that has been able to cover the total analytical range from attomolar to nanomolar concentrations, skipping any further enhancement.[45] The biosensor consists of optical waveguides, the nanometre dimensions of which change the properties of the guided light, generating ultra-sensitive interferometric signals able to detect attomolar concentration of micro-RNA directly in urine samples from bladder cancer patients. Its miniaturised size allows for multiplex arrays formats, incorporating 20 nanosensors within the same sensor chip (10 mm width, 31 mm length).

Few optical biosensors have been devised in order to use the alternative splicing regulation route for diagnostic purposes,[6-8] probably due to the long RNA sequences and the similarity between mRNA isoforms that critically complicate the differentiation between the isoforms. Single mRNA-spliced variants have been identified and quantified in living cells by quantitative imaging.[46] This advance may enhance the understanding of pharmacogenomics, genetic diagnosis, and gene therapies. Another methodology incorporated a fragmentation process in order to adapt the length of the isoforms to the biosensor convenience and standardise the detection procedure.[47] The amplification-free methodology performed an accurate and efficient analysis of the alternative spliced isoforms from different genes and contexts in HeLa cells. In addition, the further implementation of the methodology in a multiplexed nanophotonic biosensor not only improved the sensitivity of the detection, but also would allow the simultaneous detection of an array of biomarkers with the same biosensor chip.[48]

CHALLENGES IN EPIGENETIC BIOSENSING

Despite the significant advances and the promising perspectives of these new emerging diagnostic optical biosensors for identifying cancer epigenetics, they still face several challenges. Epigenetics is a relatively young discipline and, as such, still more fundamental knowledge is needed for its translation into diagnostics as an accurate mark of cancer. Due to the immense network of different epigenetic routes, it is strictly indispensable to establish accurate and well-defined epigenetic panels to be correlated with concrete cancer profiles. These panels cannot give rise to possible misleading diagnostics and should have sufficient consistence to offer a robust diagnosis. Once this accurate definition takes place, many clinical trials have to be done in order to establish standard patterns between different individuals and set up threshold concentration that confirms the presence and spreading of a tumourigenic process. For cancer stratification, different stage profiles need to be predefined in order to know the stage and level of progression of the cancer.

On the other hand, the biosensor field is still far from meeting some of the key end-user needs. Most biosensor technologies should be focussed on matching or even improving their sensitivity levels to the conventional methodologies, without the need for sample premanipulation processes. Efforts are also focussed on the development of biosensors capable of multi-test detection and simultaneous monitoring. In terms of optical biosensor integration, new microfluidic approaches need to be addressed in order to overcome the specific requirements for each epigenetic mechanism and compartmentalisation for individualised analysis inside the same sensor platform. Also, the stability, reproducibility, and life cycle should be tested and guaranteed.

CONCLUSION

The benefits of optical biosensors for the routine analysis of epigenetic biomarkers is changing the concept of cancer diagnosis. The analysis of epigenetic regulation processes is a top requisite for the careful dissemination of tumour onset and the development of personalised treatments in order to reverse the affected mechanism in each cancer and patient. The possibility for rapid,

very sensitive, and multiplex detection of different epigenetic mechanisms involved in tumour progression makes these devices an outstanding solution for cancer management. Several biosensor approaches have already been shown to fulfil the needs and overcome the challenges that conventional tools for epigenetic analysis are seeking nowadays. Further investigations are devoted to developing more robust, sophisticated, and easy-to-use devices. The combination of nanotechnology with epigenetic analysis constitutes a breakthrough in cancer theranostics, i.e. the use of one particular molecular diagnostic to guide therapeutic decisions, since it offers the possibility of not only providing a more in-depth vision of the tumourigenic process, but also paves the way for a more directed and effective route to design therapies aiming at eradicating cancer and increasing patient quality of life.

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THE JANUS-FACED ROLE OF CELL-MEDIATED IMMUNE RESPONSES IN PANCREATIC CANCER

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ABSTRACT

Pancreatic cancer (PC) still represents an unresolved therapeutic challenge due to the associated poor prognosis and the lack of responsiveness to current treatments. Surgery, followed by adjuvant therapy, is the only potentially curative treatment for PC; however, only 20% of PC patients have a potential resectable disease at diagnosis and the overall 5-year survival rate does not exceed 20%. In this context, better, more effective strategies are needed. Immunotherapy is an interesting approach for cancer treatments, but increasing evidence testifies that the immune system plays contrasting roles in both tumour elimination and tumour progression. In particular, PC is considered relatively immune resistant due to the characteristic fibrosis, the presence of immunosuppressive cells, and the compact extracellular matrix that defines the tumour microenvironment and allows for the growth of cancer cells. Despite this, there is evidence that PC cells are able to induce an anti-tumour immune response that can impact the disease course. More recently, it has become clear that PC activates both the anti-cancer immune response and the immunosuppressive effects of the immune system; therefore, for the immune-therapeutic strategies to be effective, they should involve not only the stimulation of the immune system but also the control of the immunosuppressive milieu. In this review, we discuss the dual role of immune cells in the onset and progression of PC.

Keywords: Pancreatic cancer (PC), immune response, immunotherapy, T cells, regulator T cells (Treg), natural killer (NK) cells, dendritic cells (DC), macrophages, myeloid derived suppressor cells (MDSC), mast cells (MC).

INTRODUCTION

Pancreatic cancer (PC) still represents an unresolved therapeutic challenge due to the associated poor prognosis and the lack of responsiveness to current treatments. Surgery, followed by adjuvant therapy, is the only potentially curative treatment for PC, but only 20% of PC patients have a likely resectable disease at diagnosis and the overall 5-year survival rate does not exceed 20%.[1] In this context, better, more effective strategies are needed. Several published data confirm that innate and adaptive immune cells, as well as effector molecules and pathways, can perform tumour suppressive mechanisms. However, we know that the immune system can also favour tumour progression. The Janus-faced role of the immune system is well-described by the dynamic process termed 'cancer immunoediting' (Figure 1).[2]

Anti-tumour immune responses are carried out by both innate and adaptive immune system

components, such as immune cells, cell surface molecules, costimulatory receptors, ligands, and cytokines. The innate immune cells principally involved in the fight against cancer are granulocytes, macrophages, natural killer (NK) cells, and dendritic cells (DC). These cells represent the first line of defence against invading and transformed cells, even though cancer cells can easily escape their surveillance. Other than this initial defence, the adaptive arm of the immune system, mainly represented by T and B lymphocytes, is the most important force against cancer and has the ability to recognise cells expressing foreign antigen-like neoplastic cells. Moreover, immunosuppressive cytokines and regulatory cell populations, including regulatory T cells (Treg) and tumour associated macrophages (TAM), can favour tumour growth. In this context, the action of the host's immune response against tumours can be demonstrated by the presence of immune components *in situ*. Analysis of the density, location, and functional properties of all immune cell types that may be found in pancreatic tumour tissue has allowed the identification of the components that exert an anti-tumour or a tumour-supportive role in patients (Figure 2).

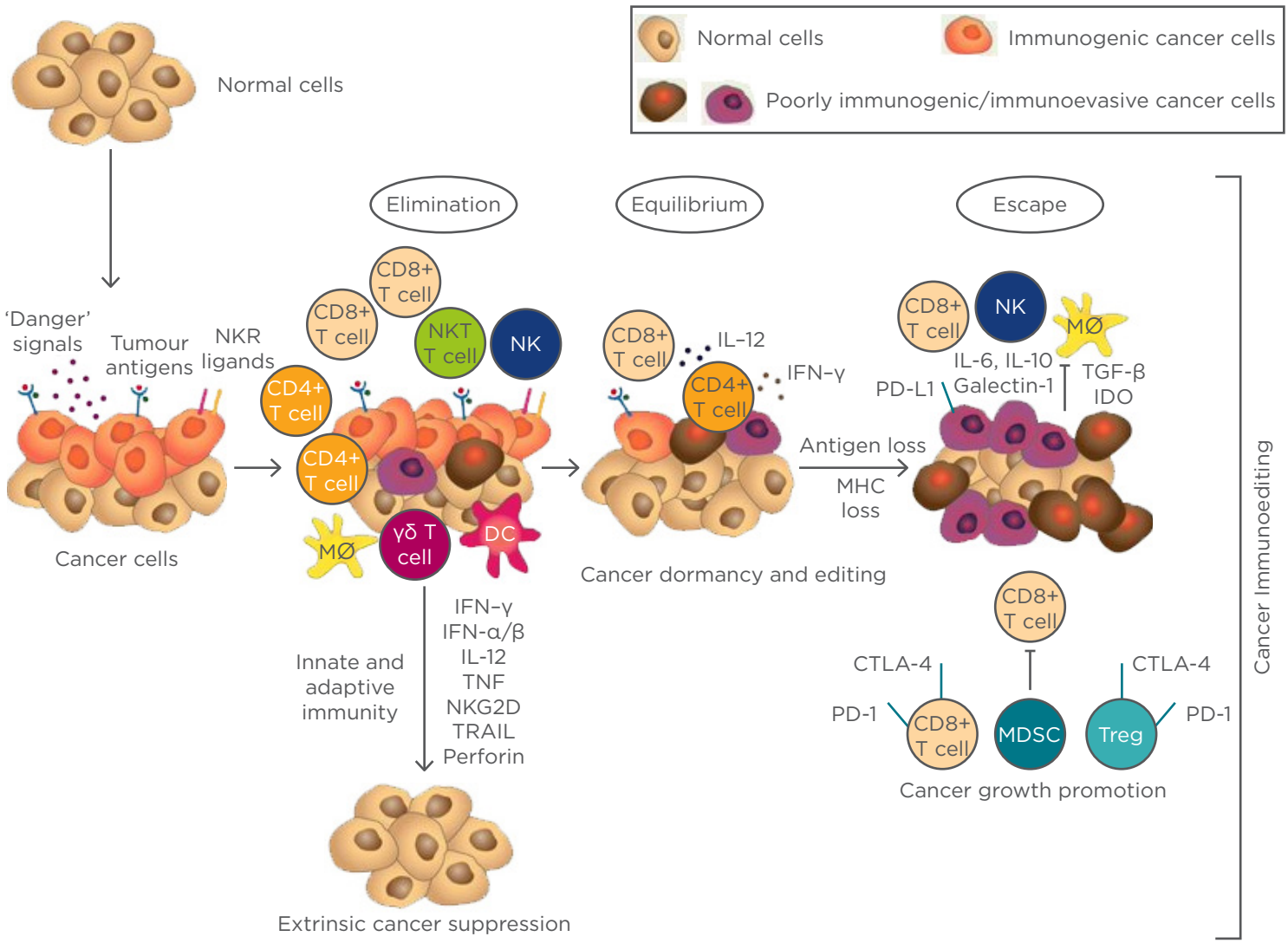

Figure 1: The three phases of the cancer immunoediting theory: Elimination, Equilibrium, and Escape. Elimination: The effector immune cells successfully eradicate the developing tumour. Equilibrium: The tumour cells and the host immune system are in balance; the adaptive immune system controls tumour growth, although shaping tumour immunogenicity. Escape: The tumour overcomes the immune system and becomes a clinically evident disease.

CTL: cytotoxic lymphocytes; DC: dendritic cell; IFN: interferon; IL: interleukin; Mø: macrophage; MDSC: myeloid-derived suppressor cell; MHC: major histocompatibility complex; NK: natural killer cell; NKT: natural killer T cell; PD-1: programmed cell death 1: TNF: tumournecrosis factor; Treg: T regulatory cell; TGF: transforming growth factor.

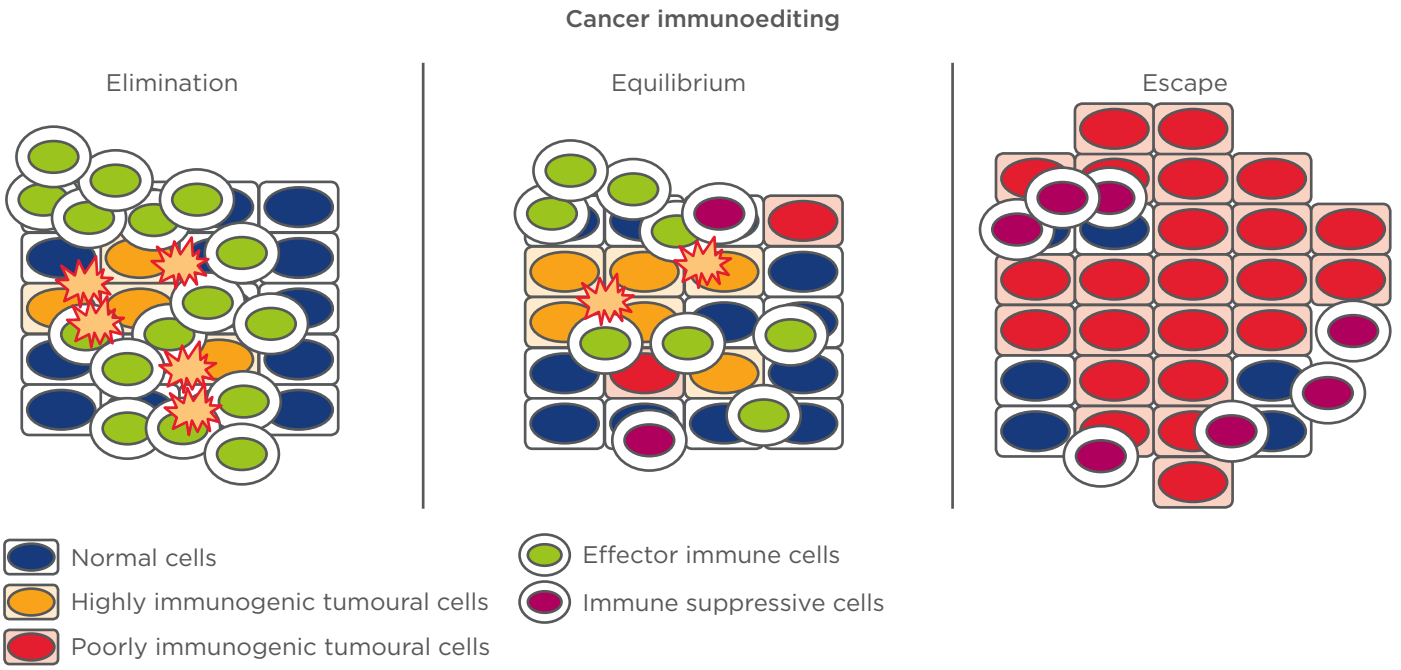

Figure 2: Diagram of cell types that interact with cancer cells in the tumour microenvironment. Immune cells that exert an anti-pancreatic cancer effect are CD8+ cytotoxic lymphocytes, natural killer T cells, CD4+ T helper (Th1, Th9, Th17), natural killer cells, and dendritic cells. Cells with a pro-pancreatic cancer role are Th2, Th22, T regulatory cells, myloid-derived suppressor cells, tumour-associated macrophages, cancer-associated fibroblasts, and mast cells.

From an immunological perspective, PC is considered an incredibly peculiar cancer type. It is well known that inflammation drives PC development and progression; >90% of all pancreatic adenocarcinomas carry an activating mutation in the RAS oncogene, which drives an inflammatory programme.[3] In addition, chronic inflammation (pancreatitis) is a documented risk factor for PC development.[4] Although often characterised by a marked leukocyte infiltration,[4] levels of intratumoural effector immune cells are limited in PC compared to other cancers.[5,6] The lack of effective immunity accompanies a massive infiltration of immune suppressive leukocytes and the presence of tumour supportive immune cells. Usually, the PC stroma encompasses the majority of the tumour mass and consists of a dynamic assortment of extracellular matrix components and non-neoplastic cells, including fibroblasts, vascular cells, and immune cells. In particular, the expansion of the tumour is associated with a substantial desmoplastic stromal reaction that changes the normal pancreatic architecture into fibrotic tissue; this fibrotic tissue primarily represents a barrier for the immune system but also interacts with neoplastic cells, favouring its development.[7]

The PC microenvironment develops an immunosuppressive phenotype, as demonstrated by the large infiltration of Treg and myeloid-derived suppressor cells (MDSC) present at the early stage of the disease. In advanced PC, T lymphocytes (CD4+ and CD8+) are rarely found in the tumour tissue.[8] In all stages of the disease, a strong inverse correlation between MDSC and CD8+ T cells persists, suggesting that MDSC are a mediator of tumour immune suppression.[8] Nevertheless, PC is able to activate an anti-tumour immune response; firstly, the accumulation of CD8+ T cells in PC correlates with survival of patients[9] and, secondly, the PC cells can express tumour-associated antigens[10,11] that are immunogenic and able to elicit a specific immune response. For example, PC patients can show intratumoural and circulating alpha-enolase-specific T cells, and the presence of anti-alpha-enolase antibodies correlates with a significantly better clinical outcome in advanced patients treated with standard chemotherapy.[11-13] For these reasons, despite both clinical and animal models showing strong evidence for inhibition of immune function in PC, patients can be candidates for immunotherapies, involving strategies that use stimulation of the immune system in order to control the tumour immune suppressive microenvironment.

ANTI-TUMOUR IMMUNE CELLS IN PANCREATIC CANCER

Effector T Cells

CD3+ T cells recognise antigens of cancer cells and are divided into either CD8+ cytotoxic lymphocytes (CTL) or CD4+ T helper (Th) cells that recognise peptides presented by the major histocompatibility complex (MHC)-I or MHC-II, respectively. The Th cells are divided into various subsets, including interferon (IFN)-γ and tumour necrosis factor (TNF)-α producing cells (Th1), interleukin (IL)-4, IL-5, and IL-13 expressing cells (Th2), T follicular helper cells (Tfh), IL-17 expressing cells (Th17), IL-9+ (Th9), IL-22 producing cells (Th22), and Treg. In addition, CTL subsets, including Tc1, Tc2, Tc17, and regulatory CD8+ T cells, have been defined. All these T lymphocyte subsets have been investigated for their implications in cancer development and anti-tumour immunity; due to their ability to produce IFN-γ and directly kill target cells, CTL are the critical mediators of the anti-tumour response.[2]

CD3+ T infiltrating lymphocytes (TIL) have been detected in human and mouse PC tissue specimens.[8,14] Interestingly, the study of TIL distribution, density, and function in the tumour microenvironment reveals their anti or pro-tumour activity.[6] In the case of PC, TIL do not usually reach the tumour cells in a consistent number, remaining confined in the peritumoural tissue.[14] One explanation for poor CTL infiltration may be the lack of neoantigen expressed by PC cells.[15] Moreover, it can be due to the effects of the stroma and suppressor immune cells.[16]

The presence of TIL in PC was reported for the first time by Ademmer et al.[17] They documented that lymphocytes were most often confined as agglomerates in the fibrotic interstitial tissue, while rare cells could be found among the epithelial neoplastic cells. The percentage of lymphocytes was changeable between samples; however, there was a predominance of T cells with a CD45RO+ memory phenotype. Among the TIL, the memory CTL exert the major antitumour effects and their frequency in resected PC was found to correlate with survival.[18]

The role of Th cells in tumours is more complicated than the role of CTL because their protective or supporting role towards cancer cells depends on their functional profile. For example, Th2 cells take part in tumour tolerance,[6] while Th1 cells antagonise tumour growth through the production of IL-2 and IFN-γ, required for the activation and proliferation of CTL activity.[19,20] Overall, memory T cells and/or T cells with a Th1 phenotype are associated with a better prognosis.[21]

The role of Th17 cells in tumourigenesis has not been clarified. Both animal models and clinical studies have suggested functions for Th17 cells (and related cytokines) in tumour development. He et al.[22] detected Th17 cells in pancreatic tumour tissues and showed that the frequency of Th17 cells was significantly higher in the lymphocytes infiltrating the PC than the adjacent tissue; in addition, the number of Th17 cells was associated with tumour stages.[22] We have recently demonstrated that *ENO1*-specific Th17 cells have a specific anti-cancer effector function in PC patients, and that there are decreased levels of these cells in cancer compared to healthy mucosa.[12]

The recently discovered subset of Th lymphocytes, named Th9 cells, secrete IL-9 together with IL-21 and play a role in several inflammatory disorders. These cells arise from reprogrammed Th2 cells upon stimulation with IL-4 and transforming growth factor (TGF)-β.[23] Th9 cells feature potent anticancer properties,[24,25] but currently there are no data on their role in PC. Natural killer T (NKT) cells are a small subpopulation of T lymphocytes with antigen-specific T cell receptors (TCR) that recognise both self antigens and foreign antigens, providing a mechanism to identify lipid antigens that are not detected by CD4+ and CD8+ T lymphocytes. In cancer, these cells were initially considered to exert a defensive role, but in recent studies they have been found to also inhibit anti-tumour responses. It is now more clear that this dual role depends on the presence of two different subpopulations of NKT cells with distinguished functions: type I NKT cells with an invariant TCR and type II NKT cells with more variable TCR. type I NKT cells support tumour immunity through the production of IFN-γ, activating NK cells, CTL cells, and DC to produce IL-12. Conversely, Type II NKT cells, distinguished by differing TCR (recognising lipids presented by CD1d), principally obstruct tumour immunity.[26] Statistically significant lower numbers of peripheral blood NKT cells were found in patients with a variety of cancers compared to healthy subjects.[27] Nagaraj et al.[28] attempted to enhance the anti-tumour effect against PC by supplementary triggering of NKT cells *in vivo*; they established a significant expansion of

IFN-γ-producing NKT cells correlates with reduced tumour growth.

Natural Killer Cells

NK cells are innate lymphocytes known as the first-line of defence against infections and neoplasia due to their ability to exert direct cellular cytotoxicity without prior sensitisation and to secrete immunostimulatory cytokines like IFN-γ. Their ability to spontaneously kill tumour cells and to activate other immune cells highlights the importance of their role in fighting primary tumours and metastases. Apart from conventional NK cells, there is a range of tissue-resident NK cells with considerable differences in terms of their origin, development, and/or function.[29] The discovery of the diversity among NK cells, and the newly arising innate lymphoid cells (ILC), in general led to a newly proposed nomenclature; thus, conventional NK cells are classed as belonging to the Group 1 ILC, distinguished from ILC2, ILC3, and other non-NK cell ILC1 subsets by their dependence on IL-15 and their intrinsic cytotoxic capacity.[30] The scanning mechanism of NK cells is what allows them to maintain surveillance of tumour cells and virus-infected cells.

Through MHC Class I loss, a common event in PC,[31] PC cells become the target of NK cells, even if they can escape from this control system. Indeed, activating receptors, such as NKG2D, are reduced on the surface of NK cells in patients with PC and these lower levels are associated with advanced disease.[32] Higher absolute levels of NK cells in the circulation were associated with a better prognosis in a small set of 13 patients with PC,[33] indicating that the immune system, through NK cells, still exerts some control on cancer growth despite disease progression.

Dendritic Cells

DC are important for immune surveillance and play a key role in cancer immunosurveillance. Indeed, induction of an effective anti-tumour response requires the active participation of host antigen presenting cells, such as DC, responsible for the presentation of tumour-specific antigens with the ability to elicit primary immune responses. DC are divided into two subsets: myeloid CD11c+ DC (DCs1) and lymphoid CD11c− DC (DCs2), which have been shown to regulate immune responses via the polarisation of Th1, Th2, or even Th3 differentiation under the influence of cytokines produced by themselves. For this reason, some biological response modifiers used in anticancer therapy upregulate the activity of DCs1, but not DCs2 activity.[34] In patients with PC, DC presence is associated with a better prognosis,[35] even if their number and function, in particular of DCs1, is decreased and defective, limiting their ability to present foreign antigens to T cells.

IMMUNE CELLS WITH PRO-PANCREATIC CANCER ACTIVITY

Tumour-Supportive T Lymphocytes

Treg are a subset of T lymphocytes, characterised by the expression of the transcription factor forkhead box protein P3 and by the production of cytokines, such as TGF-β and IL-10. They modulate the immune system, maintain tolerance, and prevent autoimmune disease. In terms of immunosuppressive activity, Treg are able to inhibit anti-tumour immune responses and are a negative prognostic indicator in various tumours. Treg were found in greater numbers in PC tissue than in non-tumoural pancreatic stroma,[12,36] and higher levels of Treg correlated with less differentiated tumours.[37] Moreover, Treg correlate with metastasis and tumour grade, and negatively correlate with patient survival.[5,36,37]

Immunohistochemical assays on PC tissues show the presence of both Th1 and Th2 cell subpopulations, with the prevalence of the Th2 subset.[38] A recent study[39] reported that the Th2/Th1 ratio is an independent predictor of disease-free and overall survival in PC patients, suggesting the implication of Th2 in cancer promotion. The pro-tumoural effect of Th2 polarisation depends on IL-4 production that, besides reducing Th1 polarisation, has a direct immunosuppressive effect on CD8+ T cells.[40]

Th22 cells, a T helper subpopulation producing IL-22, were first described in patients with inflammatory disease but have also been identified in many tumours.[41,42] Even if the role of Th22 lymphocytes in the cancer immune response is ambiguous, IL-22 seems to play a pro-tumoural role in gastrointestinal tumours.[42] In PC, the intratumoural IL-22 levels and frequency of Th22 cells are elevated compared with the peripheral blood of patients and healthy donors.[43] Moreover, the expression of both IL-22 and IL-22R1 is elevated in tissue sections of PC.[44] Recently, we observed that IL-22-producing T cells were significantly increased in PC tissue and that this increase was positively correlated with tumour, node, and metastasis (TNM) staging and

poorer patient survival.[41] We also demonstrated that IL-22 production by intratumoural Th1/Th22 cells during PC progression may therefore neutralise the anti-tumour effect of Th1-polarised T cells, protecting the cancer cells by the proapoptotic effect of IFN-γ. Moreover, the percentage of peripheral blood Th22 cells in PC patients was significantly higher compared to age-matched healthy donors, demonstrating that monitoring circulating Th22 levels could be a good diagnostic parameter and blocking IL-22 signalling may represent a viable method for innovative anti-PC therapies.

Tumour-Associated Macrophages

Frequently, cancers are infiltrated by TAM, classically divided into two subsets, M1 and M2. M1 macrophages possess proinflammatory and tumouricidal capabilities, while M2 are specialised to suppress inflammation and repair damaged tissues. TAM seem crucial in mediating PC immune escape. Kurahara et al.[45] suggested that increased M2-type infiltration might support the lymph-angiogenesis and lymphatic metastatic spread in PC; moreover, PC patients that display a high TAM infiltration had a significantly poorer prognosis. In accordance, Ino et al.[5] showed that high CD163+ and CD204+ cell infiltration correlated with a reduced disease-free and overall survival in 212 PC patients. Recently, it has been demonstrated that the depletion of a specific extratumoural macrophage population can enhance CD8+ T-cell tumour infiltration in response to CD40 agonist immunotherapy[46] and, furthermore, Rosati et al.[47] showed that the blocking of macrophage activation leads to diminishing primary tumour growth and metastasis.

Myeloid-Derived Suppressor Cells

MDSC are a heterogeneous population of cells defined by their myeloid origin, immature state, and ability to potently suppress T cell responses. Where MDSC are missing in the normal pancreas, they are quickly recruited to the PC stroma, where they can represent >60% of the infiltrating leukocytes.[48] In a spontaneous PC mouse model, Zhao et al.[49] have shown that, in the pre-malignant lesion stage, MDSC count is increased in the lymph nodes, blood, and pancreas, and that this increment becomes greater upon tumour development. *In vitro*, MDSC were capable of suppressing T lymphocyte responses. More recently, MDSC infiltration was correlated with decreased levels of CTL and T helper cells and increased levels of Treg in the blood of mice after subcutaneous injection of the non-metastatic PC cell line, Pan02. Furthermore, MDSC were able to suppress CTL *in vitro* and induce initial cancer growth.[48] Indeed, in the KrasLSL.G12D/+; p53R172H/+; PdxCretg/+ (KPC) model of metastatic PC, MDSC correlated with cancer cells and metastases, suppressed T cell proliferation, and expressed high levels of arginase and nitrite upon stimulation.[50] Gabitass et al.[51] showed that in 46 PC patients, MDSC levels correlated with a Th2 skewing for cytokine production, in particular for IL-13 and, moreover, a high concentration of MDSC in the peripheral blood was associated with poor outcomes.

Mast Cells

The role of mast cells (MC), a type of granulocyte derived from the myeloid stem cell, in cancer is not well-defined,[18] but it is known that MC are a consistent component of the tumour microenvironment in different human cancers and, depending on the tumour, MC counts have been associated with either favourable or poor prognosis. In patients with PC, MC numbers were significantly increased in tumour tissue compared to the non-tumoural pancreas and their count correlated with lymph nodes' metastases and intratumour microvessel density; furthermore, patients with a low count of infiltrating MC tended to survive longer than those with elevated numbers.[52] More recent research,[53] conducted on 53 PC patients, showed that a massive MC infiltration was present in higher grade tumours and recurrence-free and disease-specific survival was worse in patients with high numbers of MC than those with a lower MC count. In another study, comprising 67 PC tissue samples, high infiltration of MC was confirmed as a negative predictive marker of patient survival.[54]

CONCLUSION

Immunotherapy appears to be a promising treatment for cancer, including PC.[55,56] Currently, many clinical trials for PC treatment are ongoing, based on synthetic, cellular-based, autologous and allogeneic vaccines, adoptive T-cell transfer, and combination therapies. However, many of these approaches, designed to prompt or increase the anti-tumoural immune response, failed due to the immunosuppressive microenvironment that characterises PC. Indeed, in evaluating the opportunity of immune-based therapies, it is crucial to remember that from an early stage in PC progression, the capacity of the immune system to

recognise and eliminate tumoural cells is impaired, due to the ability of PC cells to affect both arms of the immune response (immune activation and suppression) and taking into account that unspecified immunotherapeutic approaches also led to the stimulation of immune suppression. In conclusion, future concepts of PC immunotherapy should be designed based on the elimination of immune suppression. In order to perform an effective therapeutic strategy, it is necessary to increase the density of intratumoural effector T cells, decrease or inhibit suppressor immune cells and receptors, and understand the role of stromal reaction and its interaction with PC immune microenvironment.

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POST-SURGICAL STRATEGIES FOR PROSTATE CANCER: CLINICOPATHOLOGIC AND GENOMICS CRITERIA

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ABSTRACT

Radical prostatectomy is widely used as the primary modality of treatment for clinically localised prostate cancer. A considerable proportion of men will have adverse histopathologic features and could benefit from adjunctive treatments: mainly adjuvant or salvage radiation.

This review focusses on the still unanswered questions:

- How to manage the patient after radical prostatectomy? Which patients need further treatment and which ones will not progress if not treated?
- How to refine patients selection for further treatments? What is the role for clinical nomograms and biomarkers and which ones are validated?
- When is it wise to propose adjuvant radiation therapy (RT) instead of observation with or without salvage RT?
- What is the optimal timing for the use of salvage RT and how to choose patients for each approach?

This article discusses the evidence available in the current literature, providing a critical analysis of the controversies of each strategy.

Keywords: Postoperative, biomarkers, adjuvant therapy, salvage therapy.

WHAT IS THE PROBLEM?

Radical prostatectomy (RP) with pelvic lymph node dissection and external beam radiotherapy are currently considered the standard treatment options for the management of localised, intermediate, and high-risk prostate cancer (pCa).[1,2] In men treated with RP, the adverse histopathologic features, such as seminal vesicle invasion, positive surgical margins, and extraprostatic extension, are known to have a higher risk of developing biochemical recurrence (BCR), local recurrence, and clinical progression. For patients with noted adverse histopathologic features, the American Society for Radiation Oncology (ASTRO)[3] and American Society of Clinical Oncology (ASCO) guidelines suggest considering adjuvant radiotherapy (ART), which could reduce BCR, local recurrence, and clinical progression risk. On the contrary, there is conflicting evidence about improvements in metastasis-free survival and overall survival.[4-6]

The European Association of Urology (EAU) guidelines[1] clearly list two different options: immediate ART after recovery of urinary function or initial observation followed by salvage radiotherapy (SRT) in the case of BCR; the National Comprehensive Cancer Network (NCCN) guidelines[2] provide similar recommendations, but also note that observation may be appropriate after RP.

ADJUVANT RADIATION THERAPY, SALVAGE RADIATION THERAPY, AND EARLY SALVAGE RADIATION THERAPY: DEFINITIONS

ART and SRT are the main modalities for radiotherapy (RT) administration after RP. Recently, the use of early salvage radiation therapy (eSRT) post-RP has been introduced. The main key differences between ART and SRT are the timing and oncological intent, while there is no significant difference in the radiation dose and irradiated field.

Currently, ART is intended for use in patients with undetectable prostate-specific antigen (PSA) after surgery, but the timing is still debated. Some urologists define ART as RT administered within the first 3 months after RP, while others wait until full recovery of urinary function, provided that PSA is still undetectable. SRT is used in patients with evidence of BCR or local recurrence, in the absence of distant metastases. BCR is currently defined as a post-RP PSA >0.2 ng/mL, although some authors have proposed higher cut-off values (PSA >0.4 ng/mL).[7]

eSRT is intended as administration of SRT at very low levels of PSA (from 0.03–0.20 ng/mL to 0.50 ng/mL),[8] albeit the exact meaning and the biological significance of 'very low PSA' remains unclear and lacks consensus.[9] A more definitive answer to this controversy will come from the ongoing randomised studies.

ADJUVANT VERSUS SALVAGE THERAPY: WHAT IS THE EVIDENCE?

Data from both the USA National Cancer Database (NCDB)[10] and from a recent web-based survey among European urologists[11] show that ART is underutilised worldwide. Hesitancy in offering routine ART may potentially be justified by evidence seen in randomised clinical trials comparing ART with initial observation;[4-6] even if ART-treated patients had higher progression-free survival in comparison to observation, half of observed men were still free of BCR at 5 years (i.e. potentially a 50% overtreatment rate) and around one-third were free of BCR at 10 years. In these trials, there was a lack of overall and cause-specific survival improvement, with the exception of the SWOG8794 trial.[4] Thus, not offering ART could reduce the risk of overtreatment, short and long-term side effects of radiation (up to 35% and 8%, respectively),[4,12,13] which affect the genitourinary and gastrointestinal systems, and treatment expenses. However, besides the ARO trial, it might be argued that, in the SWOG and the EORTC trials, a post-surgical positive PSA was relatively frequent reflecting a dubious 'adjuvant' RT.

Notably, today the relevant questions lie in whether ART is superior to observation and eSRT in terms of cancer-specific and overall survival, and how might clinicians select between these approaches? The aforementioned trials did not provide a clear answer to this question, because SRT was not administered in the early setting as it is currently used. A retrospective study of 764 pT3pN0 patients with undetectable PSA after RP showed non-inferiority of ART to eSRT at long-term follow-up (>7 years) in terms of metastasis-free survival and overall survival.[14] Additionally the results from three currently ongoing prospective multicentre open-label trials (RADICALS, RAVES, and GETUG-17) are pending.

Current evidence for eSRT is mostly based on retrospective studies, with BCR as the primary outcome and without considering clinical progression, cancer-specific survival, and overall survival. Pfister et al.[15] reviewed 10 retrospective studies related to eSRT, and concluded that eSRT had good biochemical relapse-free survival of 71% (48.0–81.8%) at 5-year follow-up. Of note, the authors discovered an almost linear association of lower PSA values at eSRT and improved biochemical relapse-free survival, with an optimal PSA threshold of roughly 0.50 ng/mL (or even lower, according to Siegmann et al.[16]). Unfortunately, no direct comparison between ART and SRT was made. These results were confirmed by Stish et al.,[17] who also reported a strong association between lower PSA level and distant metastasis, cancer-specific death, and overall survival.

Briganti et al.[18] investigated a large multi-institutional cohort of 890 pT3pN0, R0/1 pCa men who underwent ART or observation with or without eSRT (PSA <0.50 ng/mL). With regard to recurrence-free survival, the results demonstrated the non-inferiority of ART to eSRT at 2-year and 5-year follow-up. Fossati et al.[14] confirmed comparable effectiveness between ART and eSRT in terms of overall survival and metastasis-free survival in a similar multi-institutional cohort of 510 patients at almost 8-years follow-up.

THE DECISION-MAKING AFTER RADICAL PROSTATECTOMY: CONVENTIONAL TOOLS

The first instrument that was predictive of BCR after RP, brachytherapy, or external radiotherapy, was developed by D'Amico et al.[19] in 1998, stratifying men on the basis of pretreatment PSA level, biopsy Gleason score, and clinical T stage. Additionally, a study performed at the Mayo Clinic found that this D'Amico stratification was able to predict not only BCR but also clinical progression and survival.[20] A different way of addressing the problem is to summarise the most important clinical variables into a scoring system. The most relevant of these are the Cancer of the Prostate Risk Assessment post-surgical score (CAPRA-S),[21] the Stephenson postoperative nomogram,[22] and the new postoperative nomogram proposed by Dell'Oglio et al.[23] specifically aimed at men already experiencing BCR. Although these clinically based nomograms (Table 1) are often used in decision-making for patients after surgery, none of them have specifically been validated for identifying which patients will benefit from ART rather than observation with or without SRT.

Abdollah et al.[24] developed a risk score based on several histopathological adverse features (positive margins, extracapsular extension, seminal vesicle invasion, pT4 stage, nodal invasion) in patients who underwent RP and extended lymph node dissection. Despite several limitations (inclusion of node positive patients [37.7%]; unstandardised use of both ART and hormonal therapy [41.6% of patients, with an uneven distribution between the ART and non-ART groups]; non-separation of pT3b from T4), the study provided some valuable insights. Most notably, ART significantly improved overall survival and cancer-specific survival only in patients with at least two risk factors out of pGS ≥8, Stage pT3b/4, and positive lymph node count >1.

With regard to SRT, Tendulkar et al.[25] recently updated a nomogram based on concurrent androgen-deprivation therapy, pathological Gleason score, extraprostatic extension, surgical margins, seminal vesicle invasion, pre-SRT PSA, and SRT dose. Not surprisingly, a collateral finding was that early SRT (for PSA level <0.50 ng/dL) after RP led to improved BCR and distant metastases survival.

Furthermore, recent studies found that the development of BCR was not sufficient to suggest patients should receive SRT, as roughly one-third (22.9–37.0%) developed clinically evident recurrence,[26,27] and only 1 out of 20 died from pCa (5.8% of a cohort of mostly low and intermediate-risk pCa patients). Using Brockman et al.'s nomogram[28] on the basis of pathological features from prostatectomy specimens (pathological Gleason score, extraprostatic extension, seminal vesicle invasion), time to BCR, preoperative PSA, PSA level at BCR, and PSA doubling time (PSADT; the last one is not strictly necessary), authors developed a predictive tool (area under the receiver operating curve [AUC]: 0.763) for pCa mortality in men with BCR. Of note, however, is that while Brockman et al.'s nomogram accuracy does not rely heavily on PSA doubling time (AUC: 0.754 without PSADT), several authors proved it to be a relevant prognostic factor for systemic progression[29,30] and cancer-specific mortality[31] in patients with BCR. There is a general agreement for administering SRT for a PSA doubling time shorter than 6 months[32] and to defer any therapy in men with a PSA doubling time longer than 12 months.[33]

Table 1: Summary of described nomograms on predictive accuracy and reported outcomes.

Nomogram	Study population	Reported outcome(s)	Predictive accuracy
CAPRA-S[21]	Post RP – clinically localised disease	pCa recurrence	CI: 0.66
Stephenson's[22]	Post RP – clinically localised disease	pCa recurrence	CI: 0.79-0.81
Dell'Oglio's[23]	BCR after RP	CSM	CI: 0.83-0.87
Tendulkar's[25]	BCR after RP in pN0 disease treated with SRT ± concurrent ADT	Metastasis BCF	CI: 0.74 (metastasis) CI: 0.68 (BCF)
Brockman's[28]	BCR after RP	CSM	CI: 0.76 (0.75 without PSADT)

ADT: androgen deprivation therapy; BCF: biochemical failure; BCR: biochemical recurrence; CI: confidence interval; CSM: cancer-specific mortality; pCa: prostate cancer; PSADT: prostate-specific antigen doubling time; RP: radical prostatectomy; SRT: salvage radiation therapy.

Currently, standard SRT is considered for a PSA value >0.50 ng/mL, but over the past several years there has been interest in lower PSA threshold for SRT administration (early SRT: radiation therapy administered for 0.20 ng/mL<PSA<0.50 ng/mL; or ultra-early salvage radiation therapy, ueSRT: radiation therapy administered for 0.01 ng/mL<PSA<0.20 ng/mL).

Among the most interesting studies, Fossati et al.[34] investigated 716 pN0 patients with undetectable postoperative PSA who underwent eSRT for PSA recurrence (i.e. two or more 0 ng/mL<PSA≤0.50 ng/mL). The authors found that each 0.1 ng/mL PSA increase was associated with an overall 3% risk increase of BCR at 5 years; patients with at least two risk factors (including pT3b/pT4 disease, pathologic Gleason score ≥8, and negative surgical margins) have a 10% risk increase of 5-year BCR for each additional 0.1 ng/mL PSA increase, while men with one or no risk factors have a 1.5% risk increase of 5-year BCR for each additional 0.1 ng/mL PSA increase. The researchers concluded that administering eSRT at the first sign of PSA rise (>0.1 ng/mL), without waiting for the 0.50 ng/mL conventional threshold defining BCR, could confer better cancer control in high-risk patients. The available evidence on very early SRT remains controversial, with studies both in support[35] and against.[36]

There is no clear role for imaging in eSRT patient selection; current EAU guidelines themselves[1] do not recommend eSRT unless there are specific concerns about systemic spread. Data using endorectal-coil magnetic resonance imaging (MRI) with contrast enhancement are contrasting; Linder et al.[37] reported an overall sensitivity of 91% and a specificity of 45% (median post-RP PSA 0.59 ng/mL) with a sensitivity of 86% in patients with PSA <0.4 ng/mL. On the contrary, Liauw et al.[38] reported a sensitivity of only 13% with PSA level <0.3 ng/mL. Thus, clear-cut application in everyday clinical practice is currently not feasible. A review by Vees et al.[39] underlined a comparable scenario for 18F-choline and 11C-acetate positron emission tomography–computed tomography (PET/CT) in the post-RP early BCR setting (55% of studies in support and 45% studies against). More promising outcomes have recently been seen with the introduction of Ga-prostate-specific membrane antigen (PSMA) PET/CT, which has proved efficacious in the evaluation of men with BCR and 0.05 <PSA<1.0 ng/mL[40,41] with a roughly 50% detection rate for PSA <0.50 ng/mL, and 28.6% detection rate for 0.20 ng/mL<PSA<1 ng/mL in 8F-choline negative patients.[42] Valuably, a refined imaging strategy may not only help to properly confirm or exclude a local recurrence but also improve its management (e.g. deliver a boost dose with RT to the suspected macroscopic relapses).

BIOMARKERS: NEWER TOOLS TO GUIDE DECISION-MAKING AFTER RADICAL PROSTATECTOMY

In the last 10 years, numerous genomic and proteomic tests have been developed in order to refine patient selection among patients with newly diagnosed pCa, post-prostatectomy patients, systemic-disseminated pCa, and hormone-refractory pCa patients, and both improve outcomes and tailor indication for possible further therapies. Genomic predictive tools look very promising in the field of outcome prediction after RP, especially when integrated into a clinical model.[43,44] Ascertaining the most reliable predictive tools remains a challenge. A large number of gene mutations and genomic alterations are involved in prostate tumour carcinogenesis. The aforementioned features are the basis used to develop stand-alone genomic classifier predictive scores, or to empower a pre-existent clinical one, and the information provides an estimate of risk of metastases after RP.[45,46] The most promising biomarkers are discussed below and summarised in Table 2.

Decipher®

Decipher® is a 22-RNA biomarker panel genomic classifier tool developed by GenomeDx Biosciences (Vancouver, Canada) to predict post-RP pCa outcomes. It is based on differential gene expression and transcription in non-recurrent and recurrent pCa. Tissue specimens from RP are processed to extract RNA and analyse over 1.4 million coding and non-coding genomic regions. Absence/presence of 22 selected markers involved in cell differentiation, proliferation, adhesion, motility, structure, cell-cycle progression, mitosis, immune modulation, and other unknown functions are used to generate a score (ranging from 0–1), known as the Genomic Classifier (GC) score.[47] The GC score can predict BCR, metastasis, and pCa-specific mortality after RP.

The GC score has also been externally validated; Ross[48] and Karnes[49] confirmed that GC score predicts metastatic progression with good accuracy.

Den et al.[50] tested GC score in the setting of post-RP radiotherapy (both ART and SRT). One hundred and eighty-eight post-RP and post-RT (in an ART or SRT setting) patients were retrospectively studied with regard to the risk of metastatic progression. Although bias was present (retrospective analysis, equivocal selection criteria for ART versus SRT, non-standardised use of ADT), GC outperformed CAPRA-S in outcome prediction; A GC score >0.6 seemed to identify a cohort of high-risk patients who most benefitted from ART (80% hazard reduction in Cox models).

Den et al.[43] evaluated GC score in a cohort of 139 patients with adverse features at RP (i.e. pT3 or positive surgical margins) compared to a clinical score based on the Stephenson nomogram, finding them equivalent in predicting BCR and metastases-free survival (AUC of 78% and 80%, respectively). These studies suggest that patients with higher GC scores could benefit from ART, even if their risk of BCR and metastatic progression is higher, while patients with lower GC scores could most benefit from observation and eSRT. The same results were achieved by Den et al.[51] investigating outcomes from 422 post-RP men with pT3 disease or positive margins.

Freedland et al.[44] analysed the predictive value of GC score for metastases risk in post-RP and SRT patients, finding that GC score performed better than the Briganti and CAPRA-S nomograms (GC AUC: 0.85 versus Briganti nomogram AUC: 0.65 and CAPRA-S nomogram AUC: 0.63). Klein et al.[52] applied GC scores to low-risk pCa (i.e., Gleason score: 6) confirmed at RP, and found higher GC scores (>0.60) in 7% of patients despite low-risk disease.

Decipher was confirmed to be a strong predictor of metastases risk progression even when applied to prostate biopsy specimens,[53] especially when used in conjunction with NCCN risk groups (C-index for NCCN alone: 0.75; for combination NCCN with Decipher: 0.88). This amount of evidence drove the NCCN guidelines to recommend Decipher to predict metastases risk in post-RP patients with adverse features (i.e. pT3 or positive surgical margins) or BCR.[2] Despite valuable and promising insights, Decipher use to predict metastases risk after SRT still requires further validation.

Phosphatase and Tensin Homolog

The phosphatase and tensin homolog (PTEN) gene is a tumour suppressor gene involved in the PI3K/AKT signalling pathway, which promotes cell survival and proliferation. Several studies identify *PTEN* as one of the most commonly mutated or deleted tumour suppressor genes in pCa (10–70% of pCa show *PTEN* loss).[54] *PTEN* deletion is predictive of shorter recurrence-free survival[55] and higher risk of cancer-related death.[56] Cancer-related death risk has been found to be higher when PTEN and TMPRSS2-ERG gene deletions are associated.[57]

Table 2: Summary of genomic assays available for post-surgical setting.

Test	Technique	Analyte	Study population	Reported outcome(s)	Indication for test
Decipher®	RNA expression oligonucleotide microarray	mRNA of 22 coding/ noncoding genes	Post-RP; adverse pathology/high-risk features	Metastasis and CSM	• pT2 with positive surgical margin • Any pT3 • Rising PSA
			BCR after RP	Metastasis and BCF	
			ART and SRT after RP	Metastasis	
PTEN	FISH or IHC	PTEN gene	Post-RP; high-risk localised disease	BCF	Not yet recommended in clinical setting
PORTOS®	RNA expression oligonucleotide microarray	24-gene signature of DNA damage-related and radiation-related genes	Post-RP treated with or without RT	Metastasis	Not yet recommended in clinical setting

ART: adjuvant radiation therapy; BCF: biochemical failure; BCR: biochemical recurrence; CSM: cancer-specific mortality; FISH: fluorescent *in situ* hybridisation; IHC: immunohistochemistry; PSA: prostate-specific antigen; RT: radiation therapy; RP: radical prostatectomy; SRT: salvage radiation therapy.

At the moment, clinical application of *PTEN* still lacks strong external validation and further evidence is required for recommendation in guidelines as a standardised biomarker.

Post-Operative Radiation Therapy Outcomes Score

Post-Operative Radiation Therapy Outcomes Score (PORTOS) is a biomarker proteomic tool based on protein expression of a panel of 24 genes involved in DNA damage response or in immune response, which predicts individual response to RT after RP. PORTOS provides a number between 0 and 1; a lower PORTOS score identifies patients who benefit less from radiation, while a higher PORTOS score identifies men most likely to benefit from RT. This feature makes PORTOS the first validated genomic test that might predict pCa response to postoperative radiotherapy, even if further validation is needed before applying in everyday clinical practice. Zhao et al.[46] confirmed the capacity of PORTOS to refine patient selection for RT. In a cohort of 196 patients, those with a higher PORTOS treated with RT experienced a significantly lower rate of metastatic progression when compared with patients who had comparable PORTOS but did not receive radiation after RP (5% and 63%, respectively, at 10-year follow-up; hazard ratio: 0.12; $p<0.0001$). Furthermore, authors reported that Decipher, the microarray version of Prolaris® (mCCP), and CAPRA-S were not able to predict the response to postoperative radiotherapy, while PORTOS does.

FUTURE SCENARIOS IN BIOMARKERS: ONGOING RESEARCH AND DEVELOPING TOOLS

Current research about predictive tools can be divided into several categories: studies addressing patients with newly diagnosed pCa, studies addressing decision-making after RP, and those to improve management of systemic-disseminated pCa and hormone-refractory disease. As for tools, we are receiving a flourishing amount of research for genomic and proteomic biomarkers, in order to develop so-called genomic and proteomic tools, both stand-alone and nested in a pre-existent clinical tool. Nevertheless, even if knowledge about pCa biology, development, and progression pathways has increased in the past few years, we still need further information to fully understand this disease and to personalise therapies for each patient. Data from the Catalogue of Somatic Mutations in Cancer (COSMIC) database show that pCa is the expression of about 80,000 DNA mutations of a discrete number of genes: most frequently, *TP53* (14%), *AR* (8%), *SPOP* (8%), *PTEN* (7%), *KMT2C* (5%), *FOXA1* (5%), *KRAS* (4%), and *KMT2D* (4%). These genes, which are usually involved in cell proliferation and survival, are thought to co-operate in pCa development, and the fact that none of them show a strong prognostic and/or predictive value in oncological outcome on its own seems to confirm this hypothesis. Thus, developing a comprehensive, affordable, but effective biomarker tool remains a challenging effort.

FUTURE PERSPECTIVES

Prospective trials allow researchers to develop a unique study protocol but may take time to develop, have long recruitment times, and the outcomes could be outdated as soon as they are ready to be published. On the other hand, retrospective studies give scientists less control over patient follow-up and treatment but outcomes can be analysed quickly, despite the potential for bias. For this reason, we believe that good-quality retrospective evidence and large multi-institutional prospective studies both have a relevant role in providing answers to the aforementioned questions, and many others to come. The trend towards a patient-tailored therapy and the idea of a potential multi-modal pCa treatment seem to be fundamental; improving the knowledge of pCa biology seems to be a major way to assess individual risk and personalise therapies.

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PROGNOSTIC AND PREDICTIVE VALUE OF FDG-PET AS AN AID IN OESOPHAGEAL CANCER MANAGEMENT

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ABSTRACT

^{18}F-fluorodeoxyglucose positron emission tomography (FDG-PET) is widely used for cancer staging before treatment and detection of recurrence during post-treatment surveillance. It is increasingly being recognised that tumour FDG uptake values may not only be prognostic, but could have predictive value to assess for treatment response during and after neoadjuvant therapy in oesophageal cancer (OC). This review focusses on the available evidence concerning the prognostic or predictive role of FDG-PET and evaluates the potential value of FDG-PET in guiding treatment decisions in OC. The correlation between pretreatment maximum standardised uptake value (SUV_{max}) and prognosis has been demonstrated by multiple studies, although the results are inconsistent and sometimes conflicting. With regard to the predictive value for FDG-PET, post-SUV_{max} after neoadjuvant chemotherapy appears to hold better promise compared to chemoradiotherapy due to the confounding effect of radiation oesophagitis. Since a number of studies have demonstrated that FDG-PET can discriminate responders from non-responders to induction chemotherapy, the predictive value of FDG-PET imaging was evaluated prospectively and the initial results of CALGB 80803 suggested that changing chemotherapy regimen based on FDG-PET response to induction chemotherapy may improve pathologic complete response rate in PET non-responders when an alternative chemotherapy is used. Furthermore, additional research has suggested that FDG-PET response after induction chemotherapy or neoadjuvant chemotherapy may enrich a patient subset who may potentially avoid subsequent surgery after chemoradiotherapy. However, the majority of reports published on FDG-PET in OC are limited to small, retrospective, and single-institutional studies. Therefore, much of the current evidence-to-date is still hypothesis-generating and would require vigorous validation before FDG-PET could become part of routine clinical practice to direct treatment decisions.

Keywords: Oesophageal cancer (OC), ^{18}F-fluorodeoxyglucose positron emission tomography (FDG-PET), chemoradiotherapy (CRT), prognosis, pathologic response.

INTRODUCTION

Oesophageal cancer (OC) is the 6th leading cause of cancer-related death with >400,000 deaths estimated annually worldwide.[1] Oesophagectomy, radiotherapy, and chemotherapy have important roles in the curative treatment of local disease.[2] ^{18}F-fluorodeoxyglucose positron emission tomography (FDG-PET) is a well-established imaging technique for initial workup and detection of recurrence after treatment in OC.[3] Moreover, FDG-PET is now widely used in the treatment response assessment during or after neoadjuvant therapy.[3-5] In this article, we review the available evidence concerning the prognostic versus predictive role of FDG-PET in OC and evaluate the potential value of FDG-PET in guiding treatment decisions.

PRETREATMENT POSITRON EMISSION TOMOGRAPHY AND PROGNOSIS

PET is a molecular imaging technique that provides images of physiologic processes. As an analogue of glucose, FDG is metabolised similarly to glucose and accumulates in most tumours in a greater amount than it does in normal tissues.[6] Since the quantity of FDG activity is associated with proliferative activity and viable tumour cell number, the most commonly used parameter of FDG uptake is the standardised uptake value (SUV) of the primary tumour.

Multiple studies have been published on the relationship between FDG-PET at time of diagnosis and prognosis in OC. Regarding the prognostic value of baseline SUV_{max} for patients receiving surgery alone, in a meta-analysis reported by Omloo et al.,[7] all eight studies suggested that high SUV is associated with worse survival in univariate analysis; however, whether SUV_{max} is an independent prognostic factor is unclear.

The prognostic value of baseline PET SUV_{max} is much more conflicting in OC patients treated with neoadjuvant chemoradiotherapy (CRT) followed by surgery. Xi et al.[8] reported that pretreatment SUV_{max} (≥5.3 versus <5.3) was significantly associated with disease-free survival in patients receiving neoadjuvant CRT. However, another prospective study from France[9] indicated that SUV_{max} did not effectively correlate with pathological response, survival, or recurrence. Similarly, Yap et al.[10] also reported that baseline SUV_{max} had no significant predictive value on survival outcomes in patients treated with trimodality therapy (TMT). However, although the predictive role of pretreatment SUV_{max} was controversial in patients who received neoadjuvant CRT, it could identify a subgroup of patients who would benefit from therapy. A large series from MD Anderson Cancer Center (MDACC), University of Texas, Houston, Texas, USA, demonstrated that the median overall survival (OS) was similar between CRT alone and TMT in patients with clinical complete response (CR) after CRT and baseline SUV_{max} <6. In contrast, TMT patients had a significantly better survival rate than patients undergoing CRT alone if baseline SUV_{max} measured ≥6.[11]

For patients treated with definitive CRT without surgery, the prognostic value of baseline SUV_{max} has also been verified in many studies. Suzuki et al.[12] reviewed 209 patients who underwent definitive CRT and found that a higher baseline SUV_{max} (≥12.7) was associated with poorer OS. A more recent study[13] from MDACC reported that baseline SUV_{max} (≥9.7 versus <9.7) was an independent prognostic factor for progression-free survival. Atsumi et al.[14] also indicated that a higher SUV_{max} (≥10.0) was predictive of worse survival and poorer local control in OC patients receiving definitive CRT. In addition to baseline SUV_{max} of the primary tumour, the predictive value of baseline SUV_{max} of metastatic lymph nodes has also been investigated. Yap et al.[10] found that nodal SUV_{max} (≥7.0 versus <7.0) was an independent predictor of OS in patients who received definitive CRT, but not in those who received TMT.

Collectively, the prognostic value of pretreatment SUV_{max} might be influenced by treatment modality in OC patients. It should be noted that there was no uniform cut-off value for SUV and most studies used the median value as the cut-off, or set the cut-off according to receiver operating characteristic curve analysis. Moreover, previous studies have demonstrated that several pretreatment clinicopathologic parameters are associated with low FDG avidity in patients with OC, such as small tumour size and non-signet ring cell carcinoma type, which may also influence the prognostic value of SUV_{max}.[15]

POSTNEOADJUVANT TREATMENT POSITRON EMISSION TOMOGRAPHY AND PROGNOSIS

For patients treated with neoadjuvant treatment, the post-treatment PET SUV_{max} may provide additional prognostic information. In a large-scale study from Japan, FDG-PET was performed before and 2–3 weeks after completion of neoadjuvant chemotherapy in 211 OC patients.[16] The post-SUV_{max} correlated significantly with pathological response and OS. The 5-year OS rate was 62.2% for patients with post-SUV_{max} <3.5, compared to 35.1% for those with >3.5 ($p<0.001$). Multivariate analysis further identified that post-SUV_{max} was an independent prognostic factor for survival in patients who underwent neoadjuvant chemotherapy followed by surgery.

Compared with neoadjuvant chemotherapy, the predictive role of post-neoadjuvant CRT SUV_{max} was inconclusive, most likely due to the confounding effect of radiation-induced oesophagitis. Investigators from MDACC found that

post-SUV_{max} after neoadjuvant CRT was one of many independent variables to predict pathologic CR in patients with oesophageal adenocarcinoma.[17] Accordingly, van Rossum et al.[18] also reported that post-SUV_{max} was significantly associated with pathologic CR in oesophageal adenocarcinoma. However, a recent small study[19] from Japan indicated that although post-SUV_{max} was prognostic in univariate analysis, only pathological nodal staging was an independent prognostic factor in multivariate analysis.

Disappointingly, in a study reported by Piessen et al.,[9] no significant association was found between post-SUV_{max} and pathologic response or survival in patients treated with neoadjuvant CRT. Similarly, Elliott et al.[20] reported that post-SUV_{max} was not correlated with pathologic CR or complete resection, and its sensitivity for pathological nodal staging was only 10%. In a recent prospective cohort study from Ireland, 138 patients were included and PET-CR was defined as post-SUV_{max} of <4 after neoadjuvant CRT.[21] A total of 63 patients (46%) achieved PET-CR, of whom only 17 patients had pathologic CR. The sensitivity, specificity, positive predictive value, and negative predictive value of PET-CR to predict pathologic CR was 57%, 57%, 27%, and 82%, respectively. Therefore, post-SUV_{max} after neoadjuvant CRT has limited prognostic and discriminatory value for clinical application. However, it should be noted that it is rather difficult to directly compare results from different studies, because different institutions used different PET scanners with different protocols and different reconstruction algorithms.

CHANGE IN ^{18}F-FLUORODEOXYGLUCOSE UPTAKE AND PROGNOSIS

The change in SUV_{max} after neoadjuvant CRT has been studied as a prognostic factor for survival or pathologic response. Baksh et al.[22] reported that the rate of SUV change showed a significant correlation with pathologic response (r=0.178; p=0.017) in 187 patients treated with neoadjuvant CRT prior to surgery. Another small study[23] from Korea found that the decrease in SUV_{max} was a significant predictor for pathologic CR after neoadjuvant CRT in patients with oesophageal squamous cell carcinoma (SCC) and the cut-off value of decrease in FDG uptake was 72.1%. A recent report from Japan also evaluated the predictive value of a decrease in SUV_{max} in 111 patients with oesophageal SCC.[24] This study found that the decrease of SUV_{max} was significantly correlated with pathologic CR. In addition, the 5-year OS rates were 66.0% for patients with a decrease in SUV_{max} >70% and 42.2% for those with a decrease in SUV_{max} ≤70% (p=0.04). In contrast, several studies failed to find a correlation between the decrease in SUV_{max} and pathologic response or survival outcomes.[9,20,25] For example, Arnett et al.[25] concluded that change in SUV_{max} after neoadjuvant CRT was not useful for predicting pathologic response or prognosis. Overall, the prognostic value of the decrease in SUV_{max} after neoadjuvant CRT has not been definitively established.

On the other hand, the FDG-PET response after induction chemotherapy appears to be a more credible imaging marker for prognosis. In 2001, German investigators evaluated whether the reduction of FDG uptake could predict response early in the course of induction chemotherapy prior to surgery.[26] Forty patients with oesophageal adenocarcinoma were included, and they underwent FDG-PET scans at baseline and 14 days after the start of chemotherapy. When applying the cut-off value of 35% reduction of FDG uptake as a criterion for metabolic response, the sensitivity and specificity to predict clinical response was 93% and 95%, respectively. PET responders demonstrated significantly better 2-year OS rate than PET non-responders (60% versus 37%; p=0.04). This study group further prospectively validated the prognostic value of 35% SUV cut-off in 65 patients.[27] PET responders showed a high pathologic response rate (44%) with a 3-year OS rate of 70%. In contrast, prognosis was very poor for PET non-responders with a pathologic response rate of 5% (p=0.001) and a 3-year OS rate of 35% (p=0.01). Another retrospective study reported by Port et al.[28] also supported that a reduction in SUV_{max} (≥50%) after induction chemotherapy before surgical resection was significantly associated with improved disease-free survival in OC. These data provided the basis for clinical trials using FDG-PET to guide treatment decisions.

For patients treated with induction chemotherapy prior to neoadjuvant CRT, the value of FDG-PET response after induction chemotherapy was also examined. In a Phase II trial of induction and concurrent CRT with irinotecan and cisplatin followed by surgery, baseline and post-induction chemotherapy FDG-PET scans were performed in 55 patients.[29] Using 35% as the cut-off point, PET responders showed a significantly higher rate of pathologic CR rate than non-responders (32% versus

4%; p=0.009). Moreover, PET responders had a remarkably improved progression-free survival. Consistently, van Rossum et al.[30] found similar results in oesophageal adenocarcinoma. The SAKK 75/02 trial[31] also revealed that the decrease in SUV_{max} after induction chemotherapy correlated well with pathologic response, with a sensitivity of 68% and a specificity of 52%. However, due to the limited number of patients, differences in survival between PET responders and non-responders failed to reach significance in this study.

Less has been published regarding the prognostic value of FDG-PET response to induction chemotherapy in patients undergoing induction chemotherapy prior to definitive CRT. In a relatively small study reported by Ishihara et al.,[32] 16 OC patients received FDG-PET scans before and 12–24 days after induction chemotherapy. Using a cut-off value of 55% for SUV_{max} reduction rate, the 1-year OS rate was 100% for PET responders versus 60% for non-responders. However, the number of patients was too limited to reach a definite conclusion in this study. Investigators from the Memorial Sloan-Kettering Cancer Center (MSKCC) New York City, New York, USA, also investigated the prognostic significance of PET response to induction chemotherapy in 52 patients with oesophageal SCC.[33] Using a pre-established cut-off value of a 35% decrease in SUV_{max}, PET responders indicated significantly more favourable 3-year OS rate than non-responders (64% versus 15%; p=0.004).

In addition to SUV_{max}, FDG-PET image texture analysis has been investigated as an emerging tool to quantify the SUV heterogeneity, which may provide a useful representation of underlying biologic tumour characteristics.[5] These proposed features, such as metabolic tumour volume, tumour longitudinal length, and total lesion glycolysis (TLG), had been assessed in the prediction of treatment response. van Rossum et al.[30] evaluated the predictive value of PET parameters before and after induction chemotherapy in 70 patients with oesophageal adenocarcinoma for the early prediction of pathologic response to neoadjuvant CRT. The results showed that the change in TLG was predictive for a poor pathologic response at a threshold of -26%, with 67% sensitivity and 84% specificity. However, Blom et al.[34] failed to repeat these results and revealed that TLG and metabolic tumour volume were not significant predictors of pathologic response to neoadjuvant treatment in OC. This discrepancy may be related to the small sample size, different tumour delineation methods, and different image acquisition protocol in these studies.

POSITRON EMISSION THERAPY-DIRECTED TREATMENT DECISION

Since multiple studies have demonstrated that FDG-PET can identify responders and non-responders to induction chemotherapy or neoadjuvant CRT, using FDG-PET imaging to guide treatment decisions has prompted interest in clinical practice. To assess the feasibility of a PET-response-guided treatment algorithm and its potential effect on prognosis, German investigators performed the MUNICON Phase II trial, including 119 patients who underwent 2 weeks of induction chemotherapy (cisplatin, fluorouracil, and leucovorin).[35] Using a predefined cut-off value of a 35% decrease in SUV_{max}, PET responders continued to receive chemotherapy for 12 weeks and then underwent surgery. PET non-responders discontinued chemotherapy and proceeded to immediate surgery. After a median follow-up of 2.3 years, PET responders demonstrated significantly longer event-free survival than non-responders (29.7 versus 14.1 months; p=0.002). In addition, major pathologic response was noted in 58% of responders versus 0% in non-responders. This prospective study confirmed the usefulness of early PET response evaluation.

Since the MUNICON trial indicated the poor prognosis for PET non-responders, the same study group conducted a subsequent MUNICON II trial intended to improve the clinical outcome of non-responders using salvage neoadjuvant CRT followed by surgery.[36] For PET non-responders, concurrent cisplatin was delivered during radiotherapy, although it was a part of induction chemotherapy regimen. For 23 non-responders, major pathologic response (<10% residual tumour) was observed in 26% of patients, and 2-year OS rate was estimated to be 42%. No patient could achieve pathologic CR and nearly half of the non-responders had distant recurrences shortly after treatment, suggesting that distant metastases could not be controlled by continuing the same chemotherapy regimen. Therefore, although the major pathologic response rate was increased after salvage CRT, the prognosis of PET non-responders remained rather poor. Overall, these two MUNICON trials revealed that early FDG-PET response to induction chemotherapy may provide valuable

information in guiding following treatment modality, such as proceeding directly to surgery or changing chemotherapy regimen in non-responders.

Based on MUNICON trials, several studies have further investigated whether the early FDG-PET response could provide useful information for choosing a chemotherapy regimen to be used during radiation.[37-39] The MSKCC group[37] retrospectively investigated the impact of changing chemotherapy regimen during radiation in PET non-responders after induction chemotherapy. Among 201 patients, 56% were PET responders (≥35% decrease in SUV_{max}) and 38 of the 88 PET non-responders changed chemotherapy regimen during radiotherapy. The median progression-free survival for PET non-responders who changed chemotherapy was significantly longer than that of PET non-responders who did not change chemotherapy (17.9 versus 10.0 months; p=0.01). Therefore, to change the chemotherapy regimen in PET non-responders may allow a significant number of patients to achieve a significant response to treatment. Of note, this PET-directed strategy needs a prospective study to confirm its benefit. A Phase II multicentre randomised trial, the Cancer and Leukemia Group B 80803 study, seeks to answer this question.[38] Patients with oesophageal adenocarcinoma were randomised to receive 4 weeks of induction carboplatin/paclitaxel or fluorouracil/oxaliplatin/leucovorin (FOLFOX) before FDG-PET reassessment. Based on the predefined cut-off value of a 35% decrease in SUV_{max}, PET responders continued on the same regimen during neoadjuvant CRT, whereas PET non-responders crossed over to an alternative chemotherapy regimen during CRT. Patients underwent oesophagectomy 6 weeks after CRT. Goodman et al.[39] recently reported the initial results of this trial. A total of 257 eligible patients were enrolled, of whom 198 patients completed surgical resection and 22.7% of patients achieved pathologic CR. For PET non-responders who switched to alternative chemotherapy during CRT, the pathologic CR rate was 15.6%. The initial results of this prospective study suggested that changing chemotherapy regimen did improve pathologic CR rate in PET non-responders.

Another important issue in OC is whether FDG-PET response could identify patients who will safely avoid subsequent surgery after CRT. However, the published reports regarding this issue have been limited to retrospective and single-institutional studies.[40-43] Monjazeb et al.[40] studied 105 OC patients receiving CRT with or without resection, of whom 31% achieved a PET-CR after CRT (SUV_{max} ≤3). Overall, the survival of TMT patients was superior to that of CRT alone. However, despite poorer baseline characteristics, patients who achieved PET-CR after definitive CRT had excellent survival outcomes equivalent to that of patients undergoing TMT. Investigators from MDACC recently published a similar analysis in 220 patients with oesophageal adenocarcinoma.[41] Different from the previous study, all patients received induction chemotherapy before CRT with or without subsequent surgery. PET-CR was defined as SUV_{max} ≤3.0 after induction chemotherapy and 48 patients (21.8%) achieved a PET-CR in this cohort. PET-CR could predict pathologic response (p=0.003) but not survival for TMT patients, whereas PET-CR was significantly correlated with OS in patients treated with CRT alone. More importantly, PET responders treated with CRT alone had comparable OS and progression-free survival as did TMT patients. Therefore, oesophageal preservation strategies could be considered for this subset of patients. In contrast, TMT patients had significantly better survival than patients receiving CRT alone among PET non-responders.

Murthy et al.[42] evaluated whether post-CRT SUV_{max} could define the clinical outcome in OC patients. This study included 272 patients, of whom 117 underwent CRT alone and 155 underwent TMT. If the post-SUV_{max} was <4.6, survival outcomes were comparable regardless of whether patients received subsequent surgery. On the contrary, if the post-SUV_{max} was ≥4.6, patients who underwent surgery had significantly more favourable survival than patients receiving CRT alone.[43] Although these aforementioned studies showed promising results, whether using PET response is sufficient to recommend against subsequent oesophagectomy after CRT remains a question that should be validated in prospective trials.

CONCLUSION

Published data demonstrate that FDG-PET has prognostic value before, during, and after neoadjuvant treatment in OC, although the results are heterogeneous. Early decrease in FDG uptake after induction chemotherapy appears to be a promising imaging marker for prognosis. Although limited data are available, FDG-PET may guide treatment decisions by identifying non-responders to induction therapy. However, the majority of

reports published on FDG-PET in OC are limited to small, retrospective, and single-institution studies. Therefore, more research focussing on standardisation of protocols and inter-institutional technique differences should be performed. On the basis of current evidence, FDG-PET should not yet be used in routine clinical practice to direct treatment decisions. More prospective multicentre trials are encouraged to further explore the role of FDG-PET.

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UNDERSTANDING THE MICROENVIRONMENT OF MELANOMA CELLS FOR THE DEVELOPMENT OF TARGET DRUG DELIVERY SYSTEMS

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ABSTRACT

Melanoma is the most aggressive and deadly form of skin cancer. The high rate of patient death is related to advanced melanoma metastasis, which usually occurs several months to years after the primary melanoma diagnosis. At an early stage, the melanoma tumour can be removed, therefore promoting a survival rate up to 99%. In this manuscript, we elucidate the tumour microenvironment factor, which is crucial for melanoma growth, proliferation, and metastasis. Melanoma is more resistant to traditional therapies, such as chemotherapy and radiotherapy; indeed, tumour-associated macrophages are often related to the worst prognosis. A better understanding of the melanoma microenvironment, including melanoma-associated fibroblasts and hypoxia-inducible factors, will enable researchers to develop drug-delivery systems with higher anticancer activity than current melanoma therapies available on the market. This review also covers macrophage targeting melanoma, such as macrophage colony-stimulating factor receptor inhibitors, C-C chemokine ligand 2 inhibitors, and vaccines combining αFAP-PE38 and melanoma associated antigens via lentiviral vectors. We also report a study using statins, which demonstrated long circulating liposome-encapsulate simvastatin reduced tumour-associated macrophage-mediated oxidative stress and production of the hypoxia-inducible factor 1α in tumours. In melanoma, xenografts may be treated with antiangiogenic agents targeting different angiogenic pathways, such as properdistatin, which selectively removes small diameter vessels and reduces the blood supply time. Sunitinib also plays a role in reducing the density of small and large diameter vessels, although it does not change the blood supply time. Considering all these factors holistically suggests that a better understanding of the melanoma microenvironment is crucial for the development of a novel and effective therapeutic approach.

Keywords: Melanoma, skin cancer, melanoma-associated fibroblast (MAF), hypoxia-inducible factors (HIF), tumour-associated macrophages (TAM), targeting drugs.

INTRODUCTION

Melanoma is one of the most common types of cancer; in the USA, it is the 7th leading cancer in women and the 5th in men. Over the last few years, the incidence of melanoma has increased substantially, especially in women. This type of cancer is the most aggressive skin cancer, with

a high rate of patient death due to advanced melanoma metastasis, which usually occurs several months to years after the primary melanoma diagnosis. This is despite a survival rate of up to 99% if a melanoma tumour is removed at an early stage.[1] Fortunately, this paradigm has changed due to advances in new drug pathways and targeting in drug delivery systems, which may have a major impact on the development of immunotherapy and target therapy for melanoma cancer. The drugs most commonly used for the treatment of the metastatic effects of melanoma are ipilimumab and vemurafenib, both approved by the U.S. Food and Drug Administration (FDA), although both therapies have their limitations. These drugs play different roles in melanoma cells; ipilimumab can achieve durable benefits to the target cells by blocking the immune suppression of T cells that is induced by cytotoxic T lymphocyte antigen 4; however, 80–85% of patients do not respond to this therapy. On the other hand, vemurafenib can achieve rapid tumour regression by targeting melanoma cells harbouring *BRAF* Val600Glu mutations. A negative side of this drug is that patients may develop drug resistance after 6 months of treatment. This manuscript presents the melanoma network environment affecting melanoma development, focussing on tissue hypoxia, macrophages, and stromal fibroblasts.[2] It is only possible to develop new drugs and strategies for melanoma therapy, prognosis, and diagnosis through a better understanding of how the tumour microenvironment can directly affect melanoma cancer progression.

SKIN AND MELANOMA MICROENVIRONMENT

The skin represents the largest organ of our body. Normal skin consists of two distinct layers: the epidermis and the dermis. The epidermis is the upper layer formed by keratinocytes, melanocytes, and Langerhans cells. Keratinocytes are the most abundant cell type in the epidermis layer and are responsible for producing the major structural protein of the skin using growth factors and keratin to maintain normal skin homeostasis. The keratinocytes also regulate and promote the proliferation of melanocytes through connexins, desmoglein-1, and E-cadherin.[3] The epidermal-melanocyte unit is normally formed by one melanocyte surrounded by 5–8 keratinocytes. Melanocytes are responsible for producing pigments that contain melanin, called melanosomes. The major risk for melanoma development is the incidence and absorbance of ultraviolet (UV) radiation; upon UV exposure, the melanosomes are transferred from melanocytes to the keratinocytes. This mechanism can avoid DNA damage caused by UV radiation.[4] In the skin there are antigen cells, namely, Langerhans cells, which are dendritic immune cells. The dermis is mostly formed by fibroblast cells, responsible for collagen production and release, and also for producing elastin; thus, maintaining the tight connection of the dermis and the epidermis through a basement membrane. In addition to fibroblast cells, there are also pericytes, adipocytes, macrophages, and vascular endothelial cells.

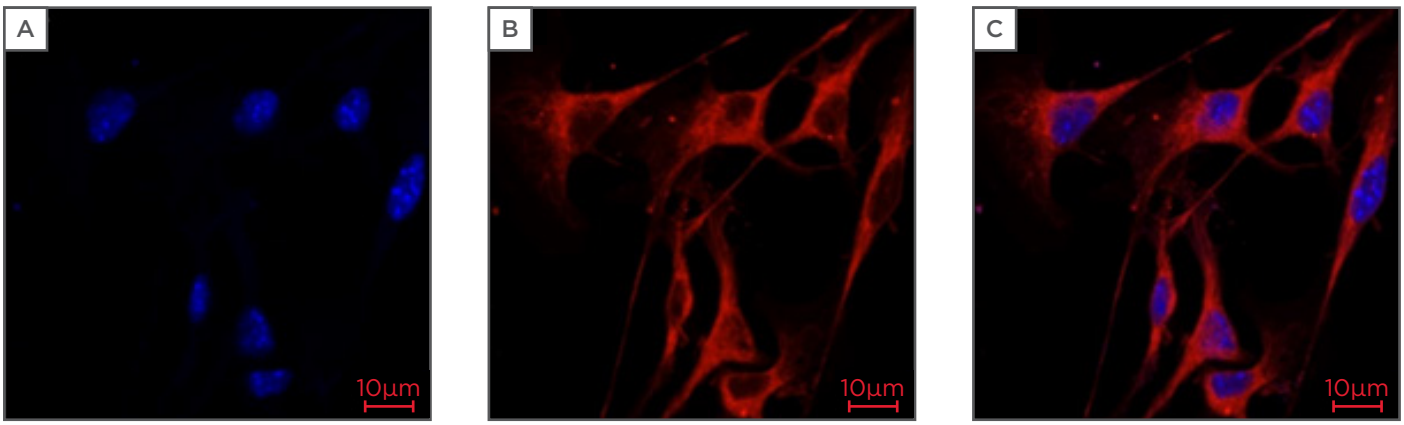

Figure 1: Confocal fluorescence images of B16 (murine melanoma cells).
The confocal images were acquired by Zeiss LSM800 Airyscan microscope (Zeiss, Oberkochen, Germany) using X63 oil immersion objective lens.
A) Stained with Hoechst to visualise the nuclei (blue); B) Cells stained with Phalloidin Rhodamine Actin to visualise cellular morphology (red); C) Multiple layers indicating both the nuclei (blue) and cellular morphology (red).

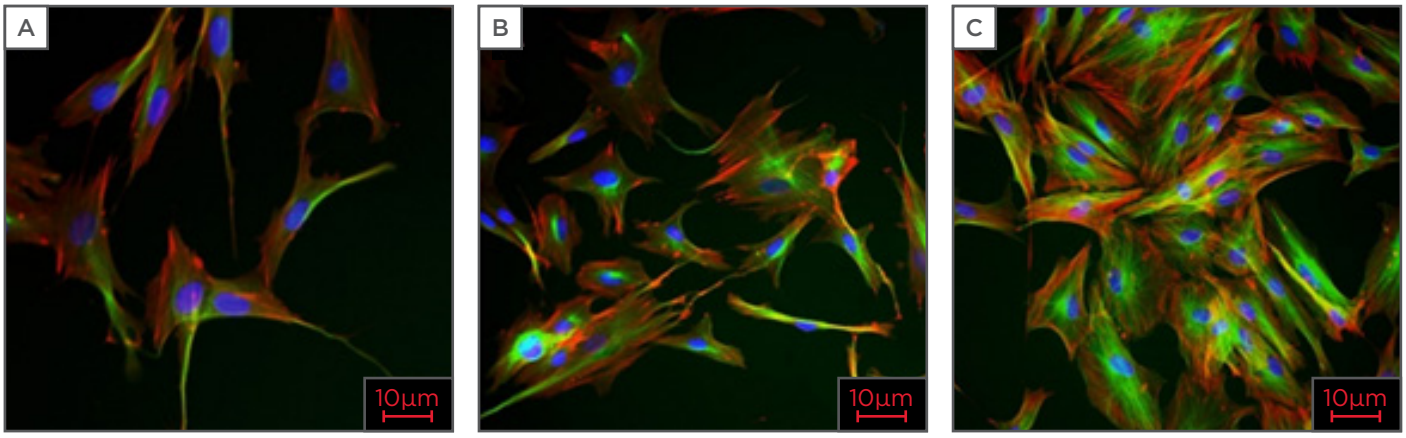

Figure 2: High content analysis of human dermal fibroblasts, magnification X20.
A) 24 hours after incubation; B) 48 hours after incubation; C) 72 hours after incubation.

The melanoma microenvironment conditions differ from the normal skin and alter as the melanoma phases progress. A radial growth phase, followed by a vertical growth phase, occur in the early stages of melanoma. The last phase, which worsens the patient's condition, occurs at the metastatic stage. When talking about the melanoma microenvironment it must be mentioned that it is highly heterogeneous and contains a variety of extracellular matrix (ECM) cells and noncancerous cells, such as fibroblasts, keratinocytes, and inflammatory cells. In this microenvironment, it is also possible to find growth factors produced by stromal cells and cancer cells. The interaction between the cancer cells and surrounding cells is very complex. The cytokines and growth factors produced by melanoma cells are responsible for recruiting many types of stromal cells, activating the tumour microenvironment, which can occur either directly or indirectly. When the growth is indirect, it affects many functions of other types of stromal cells, which play an important factor in melanoma initiation, progression, and, finally, metastasis. When the tumour is activated directly, the melanoma growth is promoted by activated stromal cells within the tumour microenvironment.[5] In Figure 1 we can observe B16 murine melanoma cells.

Deprived of adequate oxygen supply, hypoxia of the dermal environment takes place, promoting melanomagenesis.[6] While the tumour grows, it requires nutrients, oxygen, and blood; as a consequence, an uneven vasculature distribution starts to form.[5] Hypoxic tumour cells are often associated with worse prognosis, because this kind of tumour is more resistant to radiation and chemotherapies due to its peculiar biological properties and metabolic rates when compared to well-oxygenated tumours.[7] Tumour aggressiveness and hypoxia are linked, as shown in recent studies that demonstrated the treatment of tumours in mice with antiangiogenic agents can lead to tumour hypoxia, therefore enhancing the invasion and migration properties.[8,9] Tumour hypoxia and the tumour-stromal cells microenvironment can play an important role in melanoma skin cancer initiation, progression, and treatment resistance. Hypoxia-inducible factors (HIF) are present in solid tumours when the malignant tumour has a diameter >1 mm. Consequently, pockets of hypoxic regions can be visualised by immunohistochemistry with carbonic anhydrase,[9,10] primonidazole,[11] or antibodies. Normal oxygen level in the skin ranges from 1.5–5.0%. This concentration of oxygen is sufficient for the stabilisation of HIF-1α.[12] At the dermal-epidermal junction, the melanocytes are physiologically hypoxic under normal conditions; therefore, hypoxia may play an important role in melanocyte transformation. In normal skin, the nuclear HIF-1α is detected, which suggests that HIF-1α is activated in melanocytes; in other words, HIF-1α is a microphthalmia-associated transcription factor (MITF) transcription target in melanocytes.[13] MITF plays a crucial role in melanoma cancer progression, as it is a transcription factor that is involved in the regulation of genes that are related to migration, invasiveness, proliferation, survival, and metastasis of melanoma cells.

Known to induce tumour resistance to both radiation and chemotherapies, hypoxia has broad

effects on tumour fate. Hypoxia encourages growth of cells with deficient functions of tumour suppressor p53,[14] promotes genetic instability, reduces drug-induced apoptosis,[15] and favours the growth of hypoxia-tolerant tumour cell clones.[16] HIF plays an important role in melanoma tumour progression, chemotherapy resistance, and metastasis. Hypoxia can influence the metastatic progression, promoting a phenotype switch in melanoma cells from proliferative to invasive.[17] Proliferative melanoma cells, once exposed to the hypoxic microenvironment, increase the invasive potential in an HIF-1α-dependent manner and downregulate melanocytic marker expression. Melanocytes are prone to oncogenic transformation under hypoxic microenvironment due to HIF-1α stabilisation. *In vivo*, under hypoxic conditions, HIF-1α-deficient melanocytes show an ability to delay and diminish transformation capacity in tumour growth. Very aggressive melanomas can occur due to the expression of nondegradable forms of the HIF-1α protein. The permissive environment of HIF-1α may act as a tumour promoter in cells by enabling HIF-1α stabilisation at low oxygen microenvironments in the skin, where tumour cell promoters have acquired oncogenic mutations and are genetically unstable.

MELANOCYTES AND MELANOMA MICROENVIRONMENT

The microenvironment of a growing tumour is enriched in exosomes that are secreted by cancer cells. Exosomes are organelle-like, lipid-bound membrane structures shed from cell membranes into body fluids and interstitial spaces during tumour development.[18] Cancer cells can manipulate their microenvironment to optimise and achieve better growth conditions and metastasis in many ways.[19] Melanoma skin cancer is a melanocyte-originated malignant disease with high propensity to metastasise. The course of metastasis involves multiple steps, including epithelial-to-mesenchymal transition (EMT); however, the term 'EMT' may not formally be attributed to melanoma. Although melanocytes do not belong to the epithelial lineage, primary melanocytes do express E-cadherin, which is required for their contact with keratinocytes in the basal layer of the epidermis. Different to many epithelial tissues, normal melanocytes express EMT-inducing transcription factor; this is considered a predisposing factor for melanoma with a high metastatic propensity.[20] A novel therapeutic modality to target melanoma progression may be developed by an understanding of how tumour-derived exosomes contribute to the manipulation of the tumour microenvironment.[21]

HUMAN DERMAL FIBROBLASTS AND MELANOMA ASSOCIATION

During the development of a melanoma tumour, the fibroblast cells are activated by other types of stromal or tumour cells, playing an important role for tumour growth and progression. Cancer-associated fibroblasts are highly heterogeneous in terms of their functions and markers. The fibroblast-specific protein 1, commonly used as a marker for other cancers, is expressed in melanoma cells,[22] and therefore it is possible that it may also be a good melanoma-associated fibroblast (MAF) marker. However, it cannot be detected in normal skin fibroblasts.[23] Figure 2 shows human dermal fibroblast after a 24, 48, and 72-hour incubation period.

Particularly in the Caucasian population, skin cancer is by far the most common oncological malignancy. Cutaneous malignant melanoma is one of the three most common types of skin cancer; the others are non-melanocytic skin cancers, such as squamous cell carcinomas, and basal cell carcinomas. The vascularisation of the tumour occurs naturally and the cells obtain all the required nutrients to grow until reaching the size range of 2–3 mm; this is called passive diffusion.[24]

Melanoma cell growth and survival rates can be highly affected by MAF. It was shown in xenograft models that co-injection of fibroblasts cells with melanoma cells increases tumour growth.[25] In another study,[26] it was also observed that, when coculturing normal fibroblast cells in association with melanoma cells, tumour growth was promoted. This growth was reported in the early-stage of the melanoma cells, but it has very little effect on melanoma metastatic cells.[26] Some fibroblast cells can promote growth factors by overexpression of insulin, such as hepatic growth factor, basic fibroblast growth factor,[27] and insulin growth factor-1, leading to growth of biological early stages and inducing the survival rate of melanoma cells by activation of beta-catenin and mitogen-activated protein kinase (MAPK).[28] In addition, this growth factor also promotes both vascular endothelial growth factor and epithelial growth factor, both potent mitogens for the growth, proliferation, and microenvironment of melanoma cells. MAF also promote melanoma growth through glycosaminoglycan hyaluronan, also called

hyaluronate, hyaluronic acid, or HA. The synthesis of HA (HAS1 and HAS2) in MAF can activate and enhance melanoma production of platelet-derived growth factor receptor (PDGF)-CC and PDGF-AA in dermal fibroblasts and melanoma cell-derived factor stimulates hyaluronan synthesis by regulating HAS2 through p38 and PDGFR-PI3K-AKT signalling.[29] HA is one of the major components of the ECM and has been proven to promote melanoma tumour growth, angiogenesis, and metastasis.[30]

ADAM-9, a member of a family of proteases with a disintegrin and metalloprotease domain, is expressed at the tumour-stromal border. Studies *in vivo* and *in vitro* have found that stromal fibroblast-specific expression of ADAM-9 is responsible for melanoma proliferation and apoptosis, but it is controversial as to whether it exerts pro-tumour growth effects or tumour growth inhibitory effects.[31,32]

p53 is a suppressor of several types of tumours[33] and can modulate tumour growth in stromal fibroblasts in a stromal cell-derived factor 1 dependent manner.[34] Dysfunction of p53 occurs in >90% of melanoma cases.[35] In melanoma, MAF play an important role in metastasis, as the invasive potential of human melanoma cells may alter the fibroblast gene expression.[36]

Metastatic effects are caused by the spread of primary cancer cells to distant sites of the body and are attributed to the majority of cancer deaths. In melanoma metastasis, it is possible to observe sequential steps:[37,38] a) tumour cells are separated from the basement membrane, due to acquisition of an invasive phenotype, b) tumour cells can reach either a regional site or distant organs through haematogenous or lymphatic dissemination, c) tumour cells survive in a new metastatic organ such as bone, the liver, the brain, the lungs, etc.

Fibroblasts cells play an important role in melanoma angiogenesis, which consists of the formation of new blood vessels to supply nutrients for tumour growth, which is one step away from tumour cell metastasis. In a three-dimensional gel embedded with fibroblast type I collagen model and melanoma cells, normal human fibroblasts enable melanoma cells to induce angiogenesis.[39] As a consequence of melanoma metastasis, MAF can enhance melanoma invasion and angiogenesis.

TUMOUR-ASSOCIATED MACROPHAGES

The microenvironment of melanoma tumours contains many types of inflammatory cells, including mast cells, neutrophils, dendritic cells, B and T cells, and macrophages. Tumour-associated macrophages (TAM) are the most abundant cell type among immune cells; they can interfere directly with the tumour microenvironment in numerous ways, such as remodelling the ECM, increasing tumour initiation and growth, promoting angiogenesis, and suppressing antitumour immunity through the production of growth factors, cytokines, reactive oxygen and nitrogen species, and chemokines.[40-42] TAM are also linked with cancer resistance to therapies, such as immune therapy, target therapy, radiotherapy, and chemotherapy. The patient's response to antitumour therapies can be predicated by the number of macrophages; the higher the number of infiltrating macrophages, the worse the prognosis in a variety of cancers, including melanoma.[43] An inflammatory microenvironment plays an important role in every step of melanoma development and can be provided by TAM and highly inflamed tumours.

The association between melanoma and macrophages can be classified as:[2]

- M1 macrophages: Activated macrophages: the macrophage is polarised by proinflammatory factors, such as IFN-γ, and microbial agents, such as bacterial lipopolysaccharide. In this macrophage, reactive oxygen species are produced along with higher levels of nitric oxide, tumour necrosis factor α, interleukin (IL)-6, IL-12, and lower levels of the immunosuppressive cytokine IL-10. Many growth factors produced by M1 macrophages have a significant effect on promoting tumour growth and metastasis, such as IL-6 and tumour necrosis factor α.
- M2 macrophages: Alternatively activated macrophages: TAM resemble M2 macrophages and exert pro-tumour activity. M2 macrophages produce lower levels of IL-12 and a higher level of transforming growth factor (TGF)-β, IL-1 receptor antagonist (IL-1ra), IL-10, chemokine ligand (CCL)1, CCL18, and CCL22.

The WNT gene family consists of structurally related genes that encode secreted signalling proteins. These proteins have been implicated in oncogenesis and in several developmental processes, including the regulation of cell fate and patterning during embryogenesis. WNT Family Member 5A (WNT5A)

is a protein-coding gene. Linnskog et al.[44] published a study demonstrating a tumour-promoting role of increased *WNT5A* expression in malignant melanoma, sharing the ability to increase melanoma cell invasion. It reported pro-inflammatory cytokine IL-6 as a potential regulator of *WNT5A* expression. The inhibition of p38-MAPK completely blocked the IL-6-induced invasion of HTB63 cells. These findings suggest that recombinant *WNT5A* rescued the IL-6-induced invasion, indicating the inhibition of p38-MAPK activity and resulting in downstream inhibition of IL-6-induced cell invasion, related to impaired *WNT5A* expression.[44]

MELANOMA THERAPY BY MACROPHAGE TARGETING

Many approaches have been developed to target macrophages in melanoma, due to their multifunctionality and ability to promote tumour progression and metastasis. Studying the mechanisms of macrophages on tumour development has the potential to highlight possible options to treat the patient´s tumour.

Macrophage Colony-Stimulating Factor Receptor Inhibitor

Macrophage colony-stimulating factor (M-CSF) is the most potent growth factor and is highly expressed in melanoma cell lines. M-CSF is related to melanoma-associated macrophages, and activates multiple survival signals for macrophages, and binds to the M-CSF receptor (M-CSFR). Clinical trials of several M-CSFR inhibitors (M-CSFRi) for metastatic solid and advanced cancers, including melanoma, are underway. These trials are focussing on cancer therapy from the tumour microenvironment and cancer cells to tumour cells (Table 1). PLX3387, an M-CSFRi, was developed by Plexxikon Inc. (Berkeley, California, USA) and can increase the antitumour activity of *BRAF* inhibitors (BRAFi). Currently, Phase I of the trial is underway for patients diagnosed with melanoma. Anti-M-CSFR antibody, AMG 820, from Amgen (Uxbridge, UK) are under trial for advanced solid tumour therapy. The M-CSFRi, ARRAY-382, is also currently undergoing clinical trials for use in treating solid tumours.

CCL2 Inhibitor

CCL2 plays an important role in macrophage survival and differentiation, and it is the most potent chemoattractant for macrophages. For melanoma therapy, some studies indicate that anticancer therapies in combination with CCL2 targeting are useful and vital.[45] The expression of CCL2 can be decreased by the antitumour activity of BRAFi. *De novo* tumourigenesis and mouse melanoma xenograft models can achieve synergistic effects on tumour growth in combination therapy of an anti-CCL2 antibody and BRAFi.[46]

Liposome-Based Assay

Composed by bilayers of phospholipid, liposome is an artificially prepared vesicle structure. Drugs can be phagocytosed and recognised by macrophages when loaded in liposomes, providing efficient macrophage targeting. Liposomes have less toxicity than regular chemotherapy agents and can minimise the degradation of chemotherapy agents in serum.[47] Liposome-encapsulated clodronate sulphate forces the macrophages to undergo apoptosis; the removal of macrophages leads to the angiogenesis inhibition and melanoma growth in a human melanoma xenograft model.[48] Banciu et al.[49] showed that liposomal prednisolone phosphate has an antitumour growth effect on deletion of TAM in melanoma models (mouse B16).

Table 1: Summary of drugs that target macrophages.

Name	Clinical indication	Therapy combination	Pathway	Phase
PLX3397	Advanced solid tumours	Paclitaxel	M-CSFR	I
	Unresectable or metastatic melanoma	Vemurafenib		I
	Glioblastoma	Temozolomide and radiation		I/II
AMG 820	Advanced solid tumours	NO	M-CSFR	I
ARRY 382	Advanced solid tumours	NO	M-CSFR	I

M-CSFR: macrophage colony-stimulating factor receptor.
Adapted from Wang et al.[2]

CURRENT STATUS OF TARGETING STRATEGY FOR MELANOMA-ASSOCIATED FIBROBLASTS, TUMOUR-ASSOCIATED MACROPHAGES, AND HYPOXIA-INDUCIBLE FACTORS

Recently, a therapeutically effective cancer vaccine has been developed for the treatment of melanoma, which needed to generate potent antitumour immune responses and at the same time overcome the tolerance mechanism mediated by the progression of the tumour itself. A novel immunotoxin was engineered, αFAP-PE38, which targets the fibroblast activation protein-expressing fibroblast within the tumour stroma. When researchers combined αFAP-PE38 and melanoma associated antigens via lentiviral vectors (termed LV-3Ag), it exhibited greatly enhanced antitumour effects on tumour growth. The mechanism of action was described as the potential combination to modulate the immune suppressive tumour microenvironment, activating the cytotoxicity of CD8+ T cells that are able to identify and destroy the malignant cells. Combining cancer vaccines with immunotoxin could be an alternative approach for patients with advanced cancer.[44]

Statins, used for lowering cholesterol, when used in higher doses (100–500-fold higher), possess antitumour activity with regard to B16F10 murine melanoma cells. Statins can have anticancer activity due to their pleiotropic action on key regulatory molecules (small GTP-binding proteins, such as Ras, Rac, and Rho) of intracellular signalling pathways responsible for cell inflammation, proliferation, angiogenesis, oxidative stress, and apoptosis.[50] Long-circulating liposome-encapsulated simvastatin has an 85% higher anticancer activity than free simvastatin; it was reported that the antitumour activity of long-circulating liposome-encapsulated simvastatin is related to the presence of functional TAM in tumour tissue. The effect is based on the reduction of TAM-mediated oxidative stress and production of HIF-1α in tumours.[51]

Tumour hypoxia in R-18 human melanoma xenografts was reported to be treated with an antiangiogenic agent targeting different angiogenic pathways. Sunitinib may reduce the density of small and large diameter vessels whilst not changing the blood supply time, whereas properdistain selectively removes small diameter vessels and reduced the blood supply time. Both drugs were reported to inhibit angiogenesis; however, the drugs have had different effects on vascular morphology and function, and presented an extent of hypoxia in R-18 human melanoma xenografts.[52]

CONCLUSION

In this manuscript, we elucidated the cancer genetics to promote the development of new strategies for treatment of melanoma skin cancer, from the conventional therapies to target therapies. A better understanding of the melanoma tumour microenvironment can lead to a more effective therapy. Nowadays, several therapies targeting tumour microenvironments are undergoing clinical trials. The success of these trials may alter the approach of cancer therapy from tumour suppressor genes or specific protein-coding oncogenes, to targeted, less invasive, and much more effective therapies.

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CHEMOTHERAPY FOR EXTRACRANIAL GERM CELL TUMOURS IN PAEDIATRIC, ADOLESCENT, AND YOUNG ADULT PATIENTS

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ABSTRACT

Extracranial germ cell tumours (GCT) are derived from dysregulated, unipotent to totipotent, primordial germ cells and can arise from heterogeneous sites and occur across a broad age range of patients. Although healthcare professionals in the paediatric and adult medical fields collaborate closely, discrepancies in the staging system and risk-assignment used still exist. Treatment outcomes are worst in adolescent patient groups. Surgical principles have been established for treatment at initial diagnosis and during salvage therapy, as well as for the most difficult circumstances, termed desperation surgery. The development of cisplatin-containing chemotherapy marked the 1st success in GCT treatment, representing one of the major advances in the last 50 years of modern oncology. Nowadays, first-line three-drug chemotherapy regimens use cisplatin, etoposide, and either bleomycin or ifosfamide. Paediatric chemotherapy regimens typically reduce the use of bleomycin or replace cisplatin with carboplatin to decrease the levels of toxic agents in developing children. New targeted chemo-agents have been explored as potential options for refractory and relapsed GCT, as well as non-GCT malignant transformation. Here, the chemotherapy regimens currently used by paediatric and adult oncologists are described. The recent progress in targeted chemo-agents that are being used in the clinic is also discussed. Hopefully, through appropriate delivery of targeted chemo-agents, combined with well-established surgical procedures, the best outcomes of GCT for every age population can be achieved at initial diagnosis and for relapsed/refractory GCT and non-GCT transformation.

Keywords: Adolescent and young adult, chemotherapy, germ cell tumour (GCT), immune therapy, paediatric, targeted therapy, transformation.

INTRODUCTION

Extracranial germ cell tumours (GCT) can arise at heterogeneous sites, can occur across a broad age range of patients, and are treated by a variety of specialists, including paediatric oncologists, medical oncologists, urologists, or gynaecologic oncologists.[1] The common sites of extracranial GCT presentations include the gonads (testes or ovaries) and extragonadal sites (mediastinum, retroperitoneum, and sacrococcyx).[2] Due to the totipotent nature of primordial germ cells, GCT comprise a broad range of histologic subtypes, including germinomas (testicular seminomas and ovarian dysgerminomas), embryonal carcinomas, teratomas, yolk-sac tumours (formerly known as endodermal sinus tumours), and choriocarcinomas.[2] Tumours that contain various malignant histologies are termed mixed malignant GCT.[2] The outcome for adolescents with GCT is worse than for young children and adults, as reported by an institutional database and validated within the Malignant Germ Cell Tumours International Collaborative database.[2,3] As stringent evaluations, disciplined surgeries, and appropriate chemotherapies are important for successful treatment of GCT,

the under-representation of adolescents in either paediatric or adult clinical trials might have compounded the poorer outcomes in this particular age group.[2] Thus, collaborations between paediatric and adult oncologists, such as in the AO31102 study (the TIGER trial;[4] recruiting patients >14 years old), are welcomed. Current strategies for GCT treatment are comprehensively risk-based and incorporate multi-disciplinary surgical and/or chemotherapy protocols, mainly derived from the experiences of adult testicular GCT management.[5,6] However, there are significant differences in staging and risk assignment among different collaborative groups, especially between paediatric and adult oncology professionals.[1,2] Such differences can particularly affect the transitional age population, i.e. adolescents and young adults, who may receive heterogeneous GCT treatment strategies under paediatric or adult staging and risk stratification.

Surgical principles have been defined for GCT treatment at diagnosis, for resection of residual disease post-chemotherapy, as well as for relapsed or refractory disease as salvage or desperation surgery.[5,6] Chemotherapy has been known to improve long-term disease-free survival for GCT patients by 5–10% in those previously treated with actinomycin-D–based regimens, and by 50–60% due to the landmark success of cisplatin-containing regimens since the 1970s.[7] Given the toxicities associated with cisplatin-based regimens, including adverse effects on reproductive health and hearing impairment, especially in children and young adults, these regimens have evolved and diversified, particularly in the first-line treatment setting, without compromising the results.[1,2] The best salvage chemotherapy regimens have yet to be defined for relapsed/refractory GCT.

Table 1: Trichotomous categorisation of extracranial germ cell tumour by MaGIC and IGCCCG.

Systems	Low risk/good prognosis	Standard risk/intermediate prognosis	Poor risk/poor prognosis
MaGIC	T/O/EG COG Stage I Any age	T/O/EG COG Stage II–IV Age <11 years	Testicular COG Stage II–IV Age ≥11 years IGCCCG intermediate/poor
		Testicular COG Stage II–IV Age ≥11 years IGCCCG good	Ovarian COG Stage IV Age ≥11 years
		Ovarian COG Stage II–III Age ≥11 years	EG COG Stage III–IV
		Extragonadal COG Stage II Age ≥11 years	
IGCCCG (excludes clinical Stage I for prognosis assignment)	Seminoma T/O/EG No EPVM Normal STM	Seminoma T/O/EG With EPVM Normal STM	Non-seminoma Mediastinal primary or high STM‡ or EPVM
	Non-seminoma GN/RP No EPVM Low STM*	Non-seminoma GN/RP No EPVM Intermediate STM#	

*Low STM: AFP <1,000 ng/mL, HCG <5,000 U/L, LDH <1.5 x ULN; # Intermediate STM: AFP 1,000–10,000 ng/mL or HCG 5,000–50,000 U/L or LDH 1.5–10 x ULN; ‡High STM: AFP >10,000 ng/mL or HCG >50,000 U/L or LDH >10 x ULN.

AFP: alpha-fetoprotein; COG: Children's Oncology Group; EG: extragonadal; EPVM: extrapulmonary visceral metastases; GN: gonadal; HCG: human chronic gonadotropin; IGCCCG: International Germ Cell Cancer Collaborative Group; LDH: lactate dehydrogenase; MaGIC: Malignant Germ Cell Tumours International Collaborative; RP: retroperitoneal; STM: serum tumour markers; T/O/EG: testicular/ovarian/extragonadal; ULN: upper limit of normal.

In addition, current chemotherapy regimens are not effective in GCT-associated synchronous haematological malignancies, non-haematological solid malignancies, or late non-GCT malignant transformation.[8,9] However, new targeted chemo-agents, with or without immune modulation and stem cell transplantation, may improve the outcomes of these conditions. We review the current use of chemotherapy for extracranial GCT in paediatric, adolescent, and young adult patients, and highlight the differences in the approaches used by various collaborative groups.

UPFRONT CHEMOTHERAPY

Risk Assignment Before First-Line Chemotherapy

Suitable staging of newly diagnosed GCT is essential before initiating chemotherapy to ensure appropriate risk assignment. Well-known and comprehensive staging systems for GCT include the American Joint Committee on Cancer (AJCC) TNM system for testicular GCT, the International Federation of Gynecology and Obstetrics (FIGO) system for ovarian GCT, and the Children's Oncology Group's (COG) staging system for testicular/ovarian/extragonadal GCT.[1,10,11] These different staging systems may imply different chemotherapy strategies; for example, whether chemotherapy is indicated in subsets of immature teratomas differs between gynaecological and paediatric oncologists.[12,13] In this context, a recent pooled analysis of patients with ovarian immature teratomas showed similar outcomes between 98 paediatric patients (treated mostly with surgery alone) and 81 adult patients (treated with both surgery and chemotherapy), and recurrences were limited almost exclusively to patients with Grade 3, Stage III or IV tumours.[14] Table 1 illustrates two major risk assignment systems that were developed according to data on paediatric (the Malignant Germ Cell Tumors International Collaborative) and adult (the International Germ Cell Cancer Collaborative Group) GCT populations. Both systems trichotomously categorise GCT patients into low-risk/good-prognosis, standard-risk/intermediate-prognosis, and poor-risk/poor-prognosis groups.[15,16] Both risk assignment systems take into account the primary tumour sites, histologic features, metastases, and serum tumour markers, though only the Malignant Germ Cell Tumors International Collaborative considers the influence of age, especially in the youngest population. An observation-only strategy, with active surveillance prescribed after initial surgery, has been applied to patient subsets according to different definitions in clinical Stage I/II, as well as in low-risk/good-prognosis GCT groups. Although once a standard treatment, adjuvant radiotherapy directed to the retroperitoneal lymph nodes for Stage I seminomas to reduce relapse has been largely abandoned due to the increased risk of secondary malignancies.[17] Chemotherapy has been recommended for most standard-risk/intermediate-prognosis and poor-risk/poor-prognosis groups in the first-line neo-adjuvant or adjuvant setting. Incorporation of new biological agents with novel combination chemotherapies has been attempted in the context of refractory or relapsed GCT, as well as non-GCT transformation.

First-Line Chemotherapy Regimens

The standard BEP (bleomycin, etoposide, cisplatin) regimen, involving three weekly bleomycin treatments per 21-day cycle, remains the most popular GCT treatment.[18] This standard BEP regimen, given for one to four cycles mainly in adult testicular GCT studies, has produced better outcomes in randomised trials compared to regimens substituting carboplatin for cisplatin or regimens that reduce or eliminate bleomycin.[19,20] However, the paediatric PEB regimen (also bleomycin, etoposide, cisplatin), with a reduced frequency of bleomycin treatment to once every 3 weeks (i.e. once per cycle) concerning pulmonary toxicities in the developing lungs of children, has resulted in a 6-year event-free survival of 95.0% and overall survival of 95.7% in a paediatric GCT multicentre trial.[21]

In instances where bleomycin-induced pulmonary complications are a concern, the VIP/PEI (etoposide, ifosfamide, cisplatin) regimen is an appropriate alternative that has been shown to have non-inferior results (2-year failure-free survival of 63% versus 60% and overall survival of 74% versus 71%) compared to the standard BEP regimen in a randomised trial.[22] Rates of pulmonary morbidities and mortalities after second-look surgery for mediastinal non-seminomatous GCT have been decreased using neo-adjuvant VIP compared to those using the standard BEP regimen.[23]

Carboplatin has been given as a first-line, adjuvant non-radiotherapy measure to reduce the relapse rate for clinical Stage I seminoma in adult patients.[24] Adjuvant chemotherapy with the EP (etoposide

and cisplatin) regimen has been given for Stage II non-seminomatous GCT after retroperitoneal lymph node dissection in order to prevent relapse.[25] Although trials in adults have shown carboplatin to be inferior to cisplatin, the JEB (carboplatin, etoposide, bleomycin) regimen, which substitutes carboplatin (using higher doses than prescribed in the adult trials) for cisplatin, has shown 88% and 91% 5-year event-free survival and overall survival, respectively, in a multicentre childhood GCT trial, with no reports of sensorineural hearing loss.[26]

Attempts at incorporating other chemotherapeutic agents to the standard regimens have shown mixed results. Combining cyclophosphamide with PEB for paediatric GCT patients in the poor-risk category showed no clear improvement in event-free survival.[27] A single arm Phase II trial introducing paclitaxel in the TIP (paclitaxel, ifosfamide, cisplatin) regimen, adapted for outpatient administration, has generated excellent outcomes, with impressive statistics for 3-year progression-free survival (63% and 90% for poor-prognosis and intermediate-prognosis patients, respectively) and for overall survival (87% and 100% for poor-prognosis and intermediate-prognosis patients, respectively) when given as the first-line chemotherapy for GCT patients.[28] This, however, should be considered experimental until further investigation and confirmation.

Many studies, both in paediatric and adult trials, have tried acceleration or intensification of chemotherapy, as well as dose escalation after the 1st cycle, in attempts to improve the outcome of GCT. Most of these studies have not resulted in prominent improvements and were associated with unacceptable toxicities.[29] One exception is that a marginal improvement in 3-year progression-free survival (59% versus 48%) was shown in a randomised trial for the dose dense arm incorporating agents, including paclitaxel, ifosfamide, and oxaliplatin, compared to the continuing standard BEP arm in subsets of patients whose tumour markers declined only slowly. The regimen, however, failed to show a significant difference in overall survival.[30]

Table 2: Selected chemotherapy regimens for salvage of germ cell tumours.

Chemotherapy	Dosage	Duration per cycle	Results
Modified PEI (second-line with 72.6% IPFSG intermediate-to-very high risk)			
Ifosfamide Etoposide Cisplatin	2,500 mg/m² 100 mg/m² 33 mg/m²	Days 1–2 Days 3–5 Days 3–5	CR: 18.5%; PRm-: 35.4% PFS 2-year: 34.3% OS 5-year: 42.1%
TIP (second-line with previous favourable chemotherapy response)			
Paclitaxel Ifosfamide Cisplatin	250 mg/m² 1,500 mg/m² 25 mg/m²	Day 1 Days 2–5 Days 2–5	CR: 70% PFS 2-year: 65% DFS: 63%
GIP (only a previous CR to first-line chemotherapy eligible)			
Gemcitabine Ifosfamide Cisplatin	1,000 mg/m² 1,200 mg/m² 20 mg/m²	Day 1 and 5 Days 1–5 Days 1–5	CR: 54%, PRm-: 24% PFS: 51% (median FU 35 months) OS 2-year: 73%
GO (89% HDCT failure, 63% cisplatin-refractory)			
Gemcitabine Oxaliplatin	1,000 mg/m² 130 mg/m²	Day 1 and 8 Day 1	CR: 9%, PRm-: 6%, PRm+: 31% DFS: 11%
TG (all HDCT failure)			
Paclitaxel Gemcitabine	100 mg/m² 1,000 mg/m²	Day 1, 8, 15 Day 1, 8, 15	CR: 19%, PR: 13% DFS: 16%
TPG (beyond second-line)			
Paclitaxel Cisplatin Gemcitabine	100 mg/m² 50 mg/m² 800 mg/m²	Day 1 and 8 Day 1 and 8 Day 1 and 8	CR: 11%, PRm-: 39% PFS 2-year: 15% OS 2-year: 30%

CR: complete response; DFS: disease-free survival; FU: follow-up; HDCT: high-dose chemotherapy; IPFSG: International Prognostic Factor Study Group; OS: overall survival; PFS: progression-free survival; PR: partial response; PRm-: partial response with a marker normalisation; PRm+: partial response with a marker elevation.

SALVAGE CHEMOTHERAPY FOR RELAPSED OR REFRACTORY GERM CELL TUMOURS

Risk Assignment for Relapsed or Refractory Germ Cell Tumours

In a meta-analysis by the International Prognostic Factors Study Group, the 2-year progression-free survival of relapsed GCT after treatment with first-line cisplatin-containing chemotherapy ranged from 6% for the very-high-risk group to 75% for the very-low-risk group.[31] Accordingly, a comprehensive risk assignment strategy stratified patients into five prognostic subgroups on the basis of histology, primary tumour location, response to first-line therapy, tumour marker concentrations, and sites of metastases (liver, brain, and bone).[31] Clinical Stage I or good-prognosis adult patients with seminoma or non-seminoma who relapse under a surveillance strategy should be treated with 3-4 cycles of BEP or VIP, with the same recommendation under the risk assignment strategy for *de novo* metastases.[6] Similarly, PEB chemotherapy has been applied in relapsed pre-pubertal paediatric GCT patients with excellent outcomes after surveillance strategies.[32] For GCT patients who relapsed after first-line cisplatin-containing chemotherapy, it is still under debate if conventional dose chemotherapy (CDCT) is adequate, or if high-dose chemotherapy (HDCT) followed by autologous stem cell transplantation is indicated for effective salvage. An international prospective multicentre trial (TIGER study)[4] comparing initial salvage HDCT with CDCT is currently ongoing to answer this question.

Conventional Dose Chemotherapy

Traditionally, most CDCT regimens for patients with metastatic GCT relapsing after first-line chemotherapy include cisplatin and ifosfamide-containing three-drug combinations. Combinations of cisplatin and ifosfamide with either etoposide (VIP/PEI) or vinblastine (VeIP) have long been used as salvage therapy for relapsed patients after first-line BEP chemotherapy.[33,34] Recently, Phase II multicentre trials of paclitaxel or gemcitabine in combination with ifosfamide and cisplatin (TIP and GIP, respectively) have achieved higher response rates, as well as improved survival (Table 2).[35,36] A single-institute paediatric Phase II trial using a precise delivery scheme for deep regional hyperthermia in conjunction with CDCT (PEB), has shown satisfactory results, mainly for localised relapsed sacrococcygeal or retroperitoneal GCT.[37] Other new combinations, such as gemcitabine plus oxaliplatin, paclitaxel plus gemcitabine, or paclitaxel plus cisplatin plus gemcitabine, have shown responses in the most refractory subsets of GCT using observational studies (Table 2).[38-40]

High-Dose Chemotherapy Followed by Autologous Stem Cell Transplantation

A retrospective analysis of 1,594 patients with relapsed/refractory metastatic GCT showed that HDCT resulted in a higher progression-free survival and overall survival than CDCT as 1st salvage treatment.[41] Prospective studies comparing HDCT with CDCT are awaited to confirm this assumption. Carboplatin and etoposide remain the core components of HDCT for salvage of relapsed/refractory GCT, but the addition of high-dose cyclophosphamide has resulted in unacceptable toxicities.[42] Whether more HDCT cycles (when compared to single-cycle HDCT) will result in better GCT outcomes is still debatable. In this regard, a prospective randomised trial only revealed an improvement in 5-year survival that marginally favoured three cycles of HDCT over single-cycle HDCT (49% versus 39%). However, this finding could have been compounded by the higher doses of chemotherapies that were used in the single cycle arm, which also included cyclophosphamide, resulting in increased treatment-related mortality (14% in the single-cycle HDCT group compared to 4% in the three-cycle HDCT group).[42] The TICE regimen (two cycles of paclitaxel plus ifosfamide followed by three consecutive cycles of high-dose carboplatin plus etoposide), developed by the Memorial Sloan-Kettering Cancer Center, New York City, New York, USA, has induced durable remissions, even for patients with primary mediastinal GCT. This regimen can be administered on schedule more consistently and is associated with a low risk of transplant-related mortality.[43] A prospective paediatric trial has shown that complete excision of the tumour, rather than application of HDCT, is essential for the successful salvage of refractory or recurrent non-seminomatous GCT in young patients.[44]

Biologic Agents

The application of biologic agents in the treatment of GCT is in the early stages of investigation, with most studies being case series/case reports, thus, presenting only low-level evidence. Brentuximab vedotin is an antibody-drug conjugate

consisting of the chimeric antibody SGN-30 (cAC10), which is chemically conjugated to a synthetic analogue (monomethyl auristatin E) of the anti-tubulin agent dolastatin. Brentuximab vedotin targets the CD30 antigen, the expression of which is prevalent in GCT, especially in embryonal carcinoma and mixed GCT, suggesting its potential use in the treatment of GCT. In a Phase II single-centre trial, brentuximab vedotin was given to patients with CD30-expressing GCT as salvage therapy after at least two cisplatin-based regimens, including HDCT.[45] Among nine patients enrolled, there was one complete response, one partial response (>80% tumour reduction), and eight patients experienced serum tumour marker reduction after 1st cycle treatment. Five patients lost the tumour marker response after 2nd cycle treatment. It is uncertain if earlier incorporation of brentuximab vedotin will yield a better response in GCT patients.

Numerous biologic agents have been tested, mainly for chemotherapy-resistant GCT, with targets including the HER receptor family and c-KIT/stem cell factor pathway. Anti-angiogenic agents, the multitargeted tyrosine kinase, and poly (ADP-ribose) polymerase inhibitors, among others, have been investigated.[46] Until recently, no clinically meaningful activity has been shown in most trials that have investigated these targeted therapies. Nonetheless, anecdotal complete responses have been documented, including for imatinib (a small molecule inhibitor of stem cell factor receptor), in a patient with chemoresistant disseminated seminoma, and for sunitinib (a small molecule inhibitor of vascular endothelial growth factor receptor, platelet-derived growth factor receptor, stem cell factor receptor, and colony stimulation factor receptor-1) in a patient with heavily pretreated mixed embryonal carcinoma/yolk sac tumour.[47,48] Furthermore, bevacizumab, a monoclonal anti-vascular endothelial growth factor antibody, has been incorporated into HDCT schedules and showed encouraging results in heavily pretreated and refractory GCT patients.[49] Thalidomide, which has anti-angiogenic properties as well as immunomodulatory and anti-proliferative effects, has been shown to have single-agent activity in cisplatin-refractory GCT.[50]

Immune checkpoint inhibition may represent an effective strategy in poor-risk GCT since high expression of the cancer biomarker PD-L1 in GCT is correlated with worse progression-free survival and overall survival.[51] Nivolumab treatments have induced a durable response in a platinum-refractory patient with non-seminomatous GCT.[52] However, pembrolizumab treatments in a Phase II trial, with enrolment of 12 patients with metastatic non-seminomatous GCT progressed after first-line cisplatin/etoposide and/or subsequent salvage treatment including HDCT, showed no complete or partial responses, with two patients achieving stable disease at 12 and 9 weeks, respectively.[53]

High-Dose Chemotherapy with Post-Transplant Prophylactic or Pre-Emptive Targeted Chemotherapy

The efficacy of post-transplant maintenance therapy with targeted chemotherapy agents has been well demonstrated in subsets of haematologic malignancies, either as prophylactic or as pre-emptive strategies. Such an approach may be a useful strategy using CDCT regimens or biologic agents in relapsed/refractory GCT after HDCT. Our preliminary unpublished data indicate that treatment with a gemcitabine/oxaliplatin regimen, various targeted agents, and other chemotherapy agents resulted in 5/5 objective responses (2 partial and 3 complete), including three cases of relapsed/refractory GCT patients with high or very high International Prognostic Factors Study Group scores that maintained long-term disease-free survival (Table 3).

CHEMOTHERAPY FOR NON-GERM CELL TUMOUR MALIGNANT TRANSFORMATION

Derived from totipotent germ cells, GCT are capable of evolving into leukaemia or variable non-GCT solid somatic malignancies. These unique scenarios can occur in GCT patients from any primary site, although it is most common in cases of non-seminomatous GCT arising from the mediastinum.[54,55] Although chemotherapeutic agents for GCT, including etoposide/cisplatin, may effect leukaemogenesis, reported GCT-associated haematologic malignancies have evolved concurrently with, or shortly after, the GCT diagnosis.[8,55] GCT-associated haematologic malignant cells are thought to derive from GCT and spread into the blood, bone marrow, and extramedullary sites.[55] The pathology of GCT-associated haematologic malignancies is quite distinct; acute megakaryoblastic leukaemia and myelodysplastic syndrome are the most common pathologies, but malignant histiocytosis/histiocytic sarcoma are relatively over-represented.

Table 3: Stem cell therapy combined with conventional dose chemotherapy and target therapy for salvage.

Pt#/Age/Sex	Initial diagnosis	First-line	Salvage status	Salvage treatment	Outcome after salvage
No.1/24 Y/M	Med-MGCT	BEPx4-ST	MH/HS	tha/CHOPx6-PBSCT-tha	DOD 2 years
No.2/25 Y/M	Med-MGCT	ST-BEPx4-EPx2-RT	Med/Skull base relapse	TICE-tha	NED >3.5 years
No.3/17 Y/M	UP-YST (Bo/LN/Lu)	BEPx4	Refractory (Bo/LN/Lu)	TICE-tha-GO-pazopanib	DOD 1 year 10 months
No.4/15 Y/F	RP-YST	ST-BEPx4	RP relapse	EPx2-TICE-ST	NED >2.5 years
No.5/24 Y/M	T-MGCT (LN/Lu)	BEPx4-ST	Refractory	TICE-GOx4-ST-GOx2	NED >2 years
No.6/24 Y/M	T-EC (Bo/LN)	ST-BEPx4	Refractory (Bo/Li/LN/Lu)	TICE-GOx3-BV	DOD 9 months

BEP: bleomycin etoposide cisplatin regimen; Bo/Li/LN/Lu: bone/liver/lymph node/lung metastases; BV: brentuximab vedotin; DOD: died of disease; EP: etoposide cisplatin regimen; F: female; GO: gemcitabine oxaliplatin regimen; M: male; Med-MGCT: mediastinal malignant mixed germ cell tumour; MH/HS: malignant histiocytosis/histiocytic sarcoma; NED: no evidence of disease; Pt#: patient number; PBSCT: peripheral blood stem cell transplant; RP-YST: retroperitoneal primary yolk sac tumour; ST: surgical treatment; tha/CHOP: daily thalidomide plus cyclic cyclophosphamide adriamycin oncovin prednisolone regimen; tha: thalidomide regimen; T-EC: testicular pure embryonal carcinoma; TICE: paclitaxel ifosfamide carboplatin etoposide regimen; T-MGCT: testicular malignant mixed germ cell tumour; UP-YST: unknown primary yolk sac tumour.

The clinical course of GCT-associated haematologic malignancies is very aggressive, with patients either dying before treatment, not responding to antileukaemic therapy, or achieving only short remissions.[8] The prognosis is extremely poor, with patients almost invariably dying within 16 months (median survival: 5 months) after diagnosis. Two large retrospective studies involving patients with therapy-related myeloid malignancies showed that allogeneic stem cell therapy resulted in a 22% 5-year and 35% 3-year overall survival.[56,57] For patients who have achieved remission after antileukaemic therapy, immediate allogeneic stem cell transplantation should be considered, as case reports of such an approach showed efficacy in GCT-associated acute megakaryoblastic leukaemia and myelodysplastic syndrome.[58,59] A patient with mediastinal non-seminomatous GCT-associated malignant histiocytosis/histiocytic sarcoma experienced much longer survival and a good quality of life with the use of thalidomide plus CHOP (cyclophosphamide, adriamycin, vincristine, prednisolone) chemotherapy, followed by alemtuzumab-containing reduced-intensity matched unrelated peripheral blood stem cell transplantation (Table 3).[60]

GCT-associated somatic malignant transformations include various carcinomas and sarcomas. Aggressive surgery is the most important treatment for curative intent.[61] Among patients with unresectable GCT-associated primitive neuroectodermal tumours, the CAV-IE chemotherapy regimen (cyclophosphamide, doxorubicin, and vincristine alternating with ifosfamide plus etoposide), originally used for the Ewing's sarcoma family of tumours, may result in better outcomes.[62] However, neither GCT-type regimens nor those currently in use for specific somatic malignancies (with the exception of primitive neuroectodermal tumours) have shown efficacy in improving the outcomes of unresectable non-GCT somatic malignancies.[61] Other novel strategies are needed to improve the prognosis of these particular GCT scenarios.

LATE EFFECTS OF CHEMOTHERAPY

The late effects experienced by long-term survivors after cisplatin-based regimens have been reviewed recently.[2] The ototoxicity, especially in children, may result in impaired language development or hearing loss. The nephrotoxicity may cause an irreversible decrease in renal function in men with testicular GCT. The neurotoxicity, such as paraesthesia, is more often seen in adult survivors of GCT than in children. The increased prevalence of restrictive lung disease was noted in testicular

GCT survivors. Also, the increased risk of cardiovascular disease and 2nd malignancy has been shown in testicular GCT survivors.

BIOLOGY OF CHEMOTHERAPY

Numerous genetic and epigenetic mechanisms have been implicated in GCT, such as gains of chromosome arm 12p, reciprocal loss of heterozygosity (RLOH) (arm level deletions with a compensatory reciprocal amplification), high intrinsic potential of cellular apoptotic propensity (so-called mitochondrial priming), missense *KRAS* mutations, activating *KIT* mutations (specific to seminomas only), as well as wild-type *TP53*.[63] Wild-type functional *TP53*, RLOH, and high mitochondrial priming in GCT may cause a fundamental apoptotic propensity and contribute to the extreme sensitivity of GCT to cisplatin-containing chemotherapy regimens at initial diagnosis.

Plausible causal events that lead to the development of cisplatin-resistance in GCT have been proposed, which include continued progression of RLOH copy number events and loss of pluripotency markers (such as NANOG and POU5F1), as well as acquired mutations in the XRCC2, PIK3CA, AKT, KRAS, and NRAS genes.[2,63] Targeting these events in GCT with biological therapies is yet to be explored.

FUTURE DIRECTIONS

Complex multimodal strategies are required for the successful management of GCT. It is highly advisable that GCT patients are treated in expert centres. The future goal of first-line chemotherapy for GCT is to extend the success of treatment and reduce the treatment burden so that the quality of life of GCT long-term survivors can be enhanced. Collaboration between paediatric and adult oncology professionals is essential to improve the outcome of adolescents and young adults with GCT. The development of novel chemotherapy, targeted therapy, and immune therapy, either alone or in combination, is urgently anticipated to successfully salvage relapsed and refractory GCT.

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MALIGNANT PLEURAL MESOTHELIOMA: SPOTLIGHT ON RECENT ADVANCES IN DIAGNOSIS AND TREATMENT

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ABSTRACT

Pleural malignancies constitute either primary pleural malignancies, such as malignant pleural mesothelioma (MPM), or secondary pleural tumours, either from pleural metastasis or direct extension of adjacent tumours. Mesothelioma is a rare aggressive tumour of the pleural surfaces associated with prior asbestos exposure. Mesothelioma is also a challenging disease from a diagnostic staging, and treatment perspective and is rarely cured despite multimodal treatment. With incidence continuing to rise, this disease represents a serious global problem that needs urgent attention.

This review provides an in-depth review of MPM. Recent advances in diagnostic approaches, such as imaging techniques and the role of immunohistochemistry and biomarkers, are discussed. Treatment modalities, including chemotherapy, radiotherapy, and surgery as part of a multimodal approach, are reviewed, as well as the management of malignant pleural effusions.

Keywords: Pleural mesothelioma, pleural malignancy, malignant pleural effusion (MPE), biomarkers, immunotherapy.

INTRODUCTION

Malignant pleural mesothelioma (MPM) is a rare aggressive tumour derived from the mesothelium that invades the pleura. It is a challenging disease from a diagnostic, staging, and treatment perspective and is rarely cured despite multimodal treatment. During the past 20 years, important advances in the diagnosis and management of MPM have occurred; however, much is still left to be understood about this devastating disease. In this paper we provide an in-depth review of MPM with a focus on recent advances in imaging and biomarkers.

EPIDEMIOLOGY

Prior asbestos exposure is a critical risk factor for MPM, with 80% of cases caused by occupational exposure. Environmental exposure to asbestos (erionite fibres) naturally existing in the soil of areas such as Turkey, Corsica, and Cyprus, and neighbourhood exposures in people living close to asbestos factories, have also been described as risk factors for MPM. Paraoccupational exposure of household members to asbestos from the clothes of asbestos workers, as well as exposure to ceramic refractory fibres, ionising radiotherapy, and simian vacuolating virus 40, have also been linked to MPM.[1]

With an increase in industrialisation over the last two decades, the incidence of MPM is predicted to peak during 2020–2025.[1] The World Health Organization (WHO) has estimated that 92,250 deaths occur annually from asbestos-related disease. MPM portends extremely poor outcomes, with a median survival of 9–12 months. It has a 4:1 male predominance, and women have more favourable outcomes. MPM is subdivided into four histological subtypes: epithelioid, sarcomatoid, biphasic, and desmoplastic. The sarcomatoid type of MPM is associated with the poorest prognosis, with a median survival of 4 months.[2]

DIAGNOSIS

The ability to differentiate between MPM, benign pleural tumours, and pleural metastases requires multimodal evaluation. To date, there is no single diagnostic test with adequate sensitivity and specificity for early diagnosis in asymptomatic subjects. Imaging techniques, such as computed tomography (CT), magnetic resonance imaging (MRI), and positron emission tomography (PET), play a key role in the assessment of patients with suspected MPM. Depending on the presence of pleural effusions or thickening, the extent and laterality, and the presence of calcified pleural lesions, the contribution of imaging can differ significantly, from providing diagnostic or staging information to differentiating benign from malignant pleural thickening. Radiological interpretation is more challenging in early disease with minimal or absent pleural thickening, and staging can be difficult due to the heterogeneous growth patterns of MPM.

Ultrasound

Contrast-enhanced thoracic ultrasound can quantify pleural effusions or thickening, identify nodules on the pleura or hemidiaphragm, and evaluate their degree of vascularisation. Pleural-based lesions, pleural thickening >1 cm, nodular pleural thickening, and diaphragmatic nodules have >95% specificity for malignancy. The low sensitivity of 42% for differentiating malignant from benign disease, and wide interoperator variability, however, makes further evaluation of non-specific findings imperative, particularly with negative cytology from thoracentesis.[3]

Computed Tomography

The features on CT scans that distinguish MPM from metastatic pleural disease include circumferential pleural thickening (pleural rind), mediastinal pleural involvement, and a pleural thickness >1 cm. While contrast-enhanced CT is the recommended initial approach for evaluation, it has a modest sensitivity (58%) and specificity (80%) for diagnosing pleural malignancy. Around 32% of patients with pleural effusion have malignancy based on histology, despite negative findings on CT.[4] CT cannot reliably differentiate MPM from pleural metastases or between MPM subtypes. CT also has a limited role in the staging of MPM, as it is suboptimal for detecting mediastinal lymph node metastases and underestimates early chest wall invasion and diaphragmatic and peritoneal involvement. This is because mesothelioma has similar tissue attenuation to nearby structures, including the chest wall musculature, diaphragm, and pericardium.[5]

Positron Emission Tomography and Positron Emission Tomography-Computed Tomography

PET cannot reliably assess the extent of local tumour invasion and cannot differentiate MPM from pleural metastasis or between histological subtypes. Moreover, PET is associated with high false-negative rates in early disease[6] and high false-positive results in tuberculous pleuritis, parapneumonic effusions,[7] and prior pleurodesis.[8] The use of integrated PET-CT combines metabolic with anatomical information, providing improved diagnostic and staging accuracy. It is superior to MRI, PET, or CT alone for diagnosing MPM and outperforms CT and MRI in detecting intra-thoracic and extra-thoracic lymphadenopathy and extra-thoracic metastatic disease.[9] Despite its high sensitivity (88%) and specificity (93%), PET-CT does not perform well in detecting lymph node micro-metastases, specifically in N2 disease.[9] Surgical staging by endobronchial ultrasound (EBUS), mediastinoscopy, or oesophageal ultrasound fine needle aspiration, is recommended.[10]

Magnetic Resonance Imaging

MRI plays a significant role in pre-operative evaluation of MPM cases that show suspected local invasion on CT scans. It can detect chest wall or extrapleural invasion, such as mediastinal or diaphragmatic extension, more reliably than CT. MRI also has a sensitivity of 85% and specificity of 100% for detecting T3 disease or greater, but sensitivity drops to 23% for T2 disease or less.[11]

Pleural Biopsy

Histopathological confirmation is the gold standard for diagnosis of MPM. Therefore, obtaining adequate samples from pleural biopsies, both in quantity and quality, is essential. Current guidelines recommend at least five biopsies >10 mm of normal and visibly abnormal parietal and visceral pleura, and sub-pleural tissue.[1] CT-guided biopsy is preferred over 'blind' or closed needle biopsy, as the latter leads to inaccurate and smaller biopsy samples due to the lack of proper visualisation of the sampling point. Newer techniques, such as CT-guided cutting needle pleural biopsy, have

high sensitivity (91%), specificity (100%), and accuracy (91%) for diagnosing malignant lesions.[12] When compared to medical thoracoscopy, CT-guided Abrams' needle pleural biopsy had equal diagnostic sensitivity (88%). The low complication rates make it a safe procedure in patients with suspected MPM.[13]

Open biopsies via pleuroscopy (medical thoracoscopy) or video-assisted thoracoscopic surgery (VATS) allow for multiple, large, and deep biopsies and direct visualisation of visceral or diaphragmatic pleural involvement. They therefore have higher diagnostic yields and sensitivities (95%), allowing for more accurate staging.[14] Pleuroscopy is less invasive and can be performed under conscious sedation, while VATS requires general anaesthesia and single-lung ventilation. The more extensive approach, however, allows for combined diagnosis and treatment in one procedure. VATS with mediastinoscopy is recommended when mediastinal nodal involvement is suspected.[14]

Endobronchial Ultrasound

For nodal staging, EBUS provides an accurate diagnosis with minimal complication rates, and allows access to the hilar lymph nodes that are inaccessible with mediastinoscopy.[10,15] Some studies report the superior performance of EBUS over mediastinoscopy, with a sensitivity and negative predictive value of 59% and 57%, respectively, for EBUS, compared to 28% and 49%, respectively, with mediastinoscopy.[15]

Immunohistochemistry

Immunohistochemistry (IHC) is critical for differentiating MPM from metastatic carcinomas. It is most helpful in differentiating epithelioid MPM from pleural metastasis of epithelial malignancies, such as primary adenocarcinoma of the lung. Current guidelines recommend the use of monoclonal antibody panels[16] with at least two positive mesothelial markers and two negative carcinoma markers for the diagnosis of MPM.[1,16,17]

Table 1: Immunohistochemical markers used in the differential diagnosis between epithelioid malignant pleural mesothelioma, lung carcinoma, and other carcinomas.

Mesothelial markers			
Markers	**Sensitivity**	**Specificity**	**Diagnostic value**
Calretinin	>90%[18]	90–95%[16,18]	Essential
WT-1	70–95%[16,18]	100%[16,18]	Very useful
CK 5/6 keratins	75–100%[16,18]	80–90%[18]	Very useful
Vimentin	~60–65%[17]	75%[17]	Useful
Thrombomodulin (CD141)	~60%[17]	80%[17]	Less useful
D2-40 (podoplanin)	90–100%[16,18]	85%[16,18]	Useful
Carcinoma markers			
Markers	**Sensitivity**	**Specificity**	**Diagnostic value**
MOC31	95–100%[16,18]	85–98%[16,18]	Very useful
Ber-EP4	95–100%[16,18]	74–87%[16,18]	Very useful
Bg8 (Lewis Y)	90–100%[16,18]	93–97%[16,18]	Very useful
B72.3	75–85%[16,18]	>95%[16,18]	Very useful
Monoclonal CEA	80–100%[16,18]	>95%[16,18]	Very useful
Organ-specific			
Markers	**Sensitivity**	**Specificity**	**Organ**
TTF-1	75–85%[16,18]	High[18]	Lung adenocarcinoma
Napsin A	80–90%[16,18]	High[18]	Lung adenocarcinoma
p63 or p40	100%[16]	High[16]	Lung squamous cell carcinoma
Oestrogen receptor	NA[18]	NA[18]	Breast
Progesterone receptor	NA[18]	NA[18]	Breast
Mammoglobin	50–85%[18]	High[18]	Breast
PAX8	70–100%[16,18]	Unknown[16,18]	Renal
PAX2	80%[16,18]	Unknown[16,18]	Renal
RCC	~85%[18]	80–90%[18]	Renal
CD15 (LeuM1)	60–65%[16,18]	High[16,18]	Renal

The use of thyroid transcription factor 1 and napsin A can help differentiate epithelioid MPM from lung adenocarcinoma.[16] Other organ-specific markers can be used to exclude metastatic disease from other primary sites.[1,16,18] Commonly used IHC markers are listed in Table 1.

IHC has a limited role in sarcomatoid MPM. Sarcomatoid tumours express pan-cytokeratins, vimentin, and markers of smooth muscle differentiation, such as smooth muscle actin, but test negative for most mesothelial markers, with the exception of D2-40 and calretinin. Because of this, the diagnosis of sarcomatoid MPM requires at least two pan-cytokeratins, two non-mesothelial markers, and supporting clinical or imaging data.[1,16]

Non-Immunohistochemistry Biomarkers

There has been increasing interest in the role of biomarkers for earlier diagnosis of MPM. Their clinical application, however, is characterised by low sensitivity, specificity, and reproducibility, with variable results reported by published studies.

Serum mesothelin

The most widely used biomarker is serum mesothelin. Elevated levels of serum mesothelin are common in patients with MPM compared to patients with pleural metastases or asbestos-related benign pleural disease.[19] Conflicting data exist regarding the role of soluble mesothelin-related peptides (SMRP) as a diagnostic biomarker. While SMRP have high specificity (95%), they have sub-optimal sensitivity (32%), being negative in both sarcomatoid and in half of epithelioid subtypes, especially in the early stages.[20] Although pleural fluid SMRP biomarker performs better than its serum counterpart, its utility in pleural fluid samples limits its role as a screening tool for early disease, where pleural effusions are uncommon. Its greatest role is in monitoring treatment response, as SMRP levels correlate with tumour size and progression.[21]

Osteopontin

Osteopontin (OP) is a glycoprotein that is overexpressed in several malignancies, including MPM. Its role as a useful biomarker for diagnosis and prognosis of MPM has been studied thoroughly. Pass et al.[22] found that OP levels in tumour tissue, but not serum, were significantly elevated in MPM when compared to healthy controls with and without asbestos exposure. Moreover, both serum[23] and plasma[22] OP levels correlated with survival. Studies on the diagnostic power of OP, however, have produced conflicting results. This is because OP in serum is unstable, due to thrombin cleavage during the coagulation process, leading to unreliable results.[23] Elevated OP levels have also been associated with other malignancies, causing a low diagnostic specificity. Plasma OP, on the other hand, may have better diagnostic performance.[24]

Hyaluronan

Hyaluronan (HA), a polysaccharide expressed in high levels in the serum and pleural fluid of patients with MPM, is another established biomarker. Pleural fluid levels of >100,000 ng/mL have been recommended as a diagnostic indicator for MPM,[25] and elevated intracellular HA levels have also been associated with MPM.[26] Perhaps its greatest contribution, however, lies in its ability to differentiate between MPM and metastatic adenocarcinoma, since mesothelioma cells express high levels of intracellular HA, a feature not found in metastatic adenocarcinoma.[27] Serum HA levels are higher in patients with later or progressive stages compared to responders, suggesting that HA is a marker of progressive disease.[28]

MicroRNA

MicroRNA (miRNA) are short, non-protein coding single-stranded RNA involved in the regulation of gene expression and can contribute to either oncogenesis or tumour suppression. They are stable tissue-specific molecules that can differentiate mesothelioma from pleural metastases. Several studies have explored different miRNA expression profiles in MPM tissues, serum, and pleural fluid, using microarray profiling and quantitative real-time polymerase chain reaction. These studies have helped determine a subset of miRNA that are differentially expressed between MPM and healthy tissue. Specific miRNA for each histopathological subtype have also been identified. For example, miR-126 has been consistently shown to be downregulated in MPM tissue compared to normal pleura.[29] Despite its high sensitivity, miR-126 lacks tumour specificity since it is also expressed in other malignancies. miR-126 downregulation, in combination with established biomarkers such as mesothelin, could possibly be used for early detection of MPM. Plasma miRNA, such as miR-625-3p,[30] and two distinct serum miRNA[31] have also been validated as diagnostic markers for MPM.

SOMAscan™

Protein analysis with a 13-protein biomarker detection assay (SOMAscan™) has gained interest for diagnosing MPM, with high sensitivity (94%) and specificity (91%). Although larger studies are needed to validate its diagnostic performance, this novel technique could also identify potential targets for treatment.[32]

STAGING

Staging of MPM is based on the recommendation by the International Mesothelioma Interest Group, where TNM classification of the primary tumour, lymph node involvement, and distant metastasis is followed. The 8th edition of TNM Staging for MPM is detailed in Table 2.[33]

TREATMENT

Treatment remains the most challenging aspect of MPM because it responds poorly to chemotherapy and radiation therapy (RT), and surgery is rarely curative. A multidisciplinary approach to define best treatment strategy is preferred. Treatment is dictated by the extent of tumour invasion and pre-operative TNM staging. Unfortunately, staging is often corrected intra-operatively after direct visualisation of tumour extension.[34]

Chemotherapy and Immunotherapy

Irrespective of surgical resection, chemotherapy improves survival in MPM.[35] Combination therapy with cisplatin or carboplatin with pemetrexed or raltitrexed is first-line and confers a survival benefit when compared to cisplatin alone.[36] Several trials are currently studying immunomodulators, including the vascular endothelial growth factor inhibitor, bevacizumab,[37] and tyrosine kinase inhibitor, sunitinib.[38] Pembrolizumab, an anti-programmed cell death receptor 1 antibody, shows promising results in patients with programmed cell death ligand 1 positive MPM.[39] Randomised studies, however, are needed to confirm these results. Because mesothelin is highly expressed by mesothelioma cells, it makes a suitable target for immunomodulators. Several anti-mesothelin therapies, such as amatuximab, a chimeric monoclonal antibody,[40] and recombinant immunotoxins,[41] have shown improved response rates and overall survival in smaller studies. These drugs are currently being studied in larger, randomised clinical trials.

Radiation Therapy

While RT has not demonstrated a survival benefit in MPM, it plays an important role in palliation and symptom management. RT to large areas of the body, such as the whole hemithorax, carries significant risks of organ toxicity, especially to the lung, liver, heart, bone marrow, and oesophagus.[42] Although improved techniques and use of intensity-modulated RT (IMRT) have diminished toxicity while providing adequate radiation, RT-induced pneumonitis remains a significant problem.

For palliation, a short course of RT is often used to relieve the chest pain from chest wall invasion.[43] Prophylactic RT directed to pleural intervention sites is no longer recommended prior to thoracoscopy or thoracotomy, due to the lack of evidence showing a reduction in tumour seeding through these scars.[44] RT can be given as part of adjuvant treatment after chemotherapy and surgery to control residual microscopic disease. The large surface of the pleural space and tumour growth in the diaphragmatic creases and the lobar fissures, however, require high doses to achieve local control, which exponentially increases toxicity risk.[45] Some trials, however, report acceptable toxicity with ipsilateral RT after lung preservation surgery.[46] Ongoing studies, including the Prophylactic Irradiation of Tracts (PIT) trial, aim to identify optimal timing for post-surgical IMRT.[47]

Surgery

Surgery via extrapleural pneumonectomy (EPP) or extended pleurectomy/decortication (P/D) is used for staging and curative intent. EPP involves complete removal of the visceral and parietal pleura and the ipsilateral lung, resection of the ipsilateral pericardium and diaphragm, and dissection of mediastinal lymph nodes. P/D involves removal of the pleura and release of the lung and chest wall from constriction caused by the tumour, without pneumonectomy.[48] With either technique, the main objective is complete macroscopic and microscopic resection of all malignant growth. Unfortunately, obtaining negative resection margins is extremely difficult to achieve intra-operatively, due to the proximity to adjacent structures.

EPP is a more extensive surgery, reserved for patients who are candidates for multimodal therapy with neoadjuvant chemotherapy and post-surgical RT. While mortality is low at 5%, complications, including cardiac and respiratory failure, empyema, and bleeding, are common.[49] P/D involves fewer

complications at the expense of higher recurrence rates. It is used for patients with diffuse parietal involvement but small local visceral invasion. P/D can be performed using VATS, further reducing the morbidities associated with thoracotomy and allowing for simultaneous pleurodesis intra-operatively.[50] Several studies that aimed to determine the best surgical technique have failed. A retrospective review of 663 patients showed slightly increased survival with EPP. These findings, however, were thought to be largely due to selection bias and patient factors.[51] The MARS trial compared EPP with no EPP after neoadjuvant chemotherapy. The feasibility study could only enrol 45 patients and, even in this small cohort, survival benefit, albeit small, was in favour of no EPP.[52] A follow-up study, MARS-2, that aims to compare P/D versus no P/D is currently enrolling patients.[53]

Several studies have examined the feasibility and safety of sequential multimodal therapy using neoadjuvant chemotherapy followed by surgery and RT. While some studies report improved 90-day mortality rates, the biggest limitation has been high complication rates and feasibility, with less than half of patients successfully enrolled.[52] Currently, the most widely accepted strategy in patients with acceptable functional status and limited disease is multimodal therapy, in specialised centres only.[1]

Pleurodesis

Malignant pleural effusions (MPE) occur frequently with MPM. The diagnosis of MPE is an ominous sign of widespread metastasis and portends a grave prognosis, with median survival of 4 months. MPE can decrease quality of life due to dyspnoea, cough, and chest pain.

Treatment is tailored towards symptomatic relief with pleurodesis to prevent recurrent effusions. Chest tube pleurodesis requires drainage of the pleural effusion via chest tube insertion, followed by installation of sclerosing agents into the chest tube. Sterile talc is the most effective sclerosing agent and is therefore preferred over tetracycline, bleomycin, or doxycycline.

Table 2: 8th edition of TNM classification of malignant pleural mesothelioma from the International Mesothelioma Interest Group.[33]

Stage	Tumour extension
T1	Ipsilateral parietal or visceral pleural involvement.
T2	Ipsilateral parietal or visceral pleural involvement with invasion of either underlying lung or diaphragmatic muscle.
T3	Locally advanced, potentially resectable tumour. Involvement of ipsilateral parietal or visceral pleura with invasion of at least one of the following structures: endothoracic fascia, mediastinal fat, focal resectable soft tissue of chest wall, or nontransmural invasion of the pericardium.
T4	Locally advanced, unresectable tumour. Involvement of ipsilateral parietal or visceral pleura with invasion of at least one of the following structures: internal surface of the pericardium (with or without effusion), peritoneum, mediastinal structures (such as oesophagus, trachea, and great vessels), contralateral pleura, spine (including vertebrae, neuroforamen, spinal cord, and brachial plexus), or diffuse, unresectable invasion of the chest wall (with or without rib destruction).
N0	No regional lymph node metastasis.
N1	Ipsilateral intrathoracic lymph node metastasis (such as bronchopulmonary, subcarinal, hilar, paratracheal, paraoesophageal, aortopulmonary, peridiaphragmatic, pericardial, intercostals, or internal mammary nodes).
N2	Contralateral intrathoracic lymph node metastasis or metastasis to ipsilateral or contralateral supraclavicular lymph nodes.
M0	No distant metastasis
M1	Distant (extrathoracic, haematogenous, or non-regional lymph node) metastasis present.
Stage I	IA: T1 N0 M0 IB: T2-3 N0 M0
Stage II	T1-2 N1 M0
Stage III	IIIA: T1-3 N1 M0 IIIB: T4 N0-2 M0
Stage IV	Any T4 Any N M1

Thoracoscopic pleurodesis can be performed by VATS or pleuroscopy, where mechanical pleural abrasion precedes instillation of sclerosants into the pleural space. VATS pleurodesis is the preferred method because it allows for better distribution of talc and release of adhesions, causing complete lung expansion and improved apposition of pleural surfaces.[1] Pleurodesis should be considered in patients with life expectancies of >3 months, whose symptoms are relieved with therapeutic thoracentesis. Pleurodesis may be difficult to achieve in patients with trapped lungs or multiple loculations, as it requires the lung to expand against the chest wall with apposition of visceral and parietal pleural membranes. Pleurodesis is most effective when performed early. Small bore chest tubes (<16 Fr) are associated with reduced pain compared to large bore tubes and are equally effective in achieving pleurodesis.[54] Pleurodesis is difficult to achieve in MPM, due to lung entrapment by the tumour, the presence of the tumour in the pleural cavity, and the resistance of malignant mesothelial cells to talc pleurodesis.[55]

Placement of a tunnelled pleural catheter (TPC) should be considered for symptom relief, particularly in patients with unexpandable lung or failed pleurodesis. It is as effective at relieving symptoms as talc slurry via chest tubes, and can sometimes lead to spontaneous pleurodesis.[56,57] Placement of the TPC can be carried out at the bedside or as an outpatient procedure. Complications include infection, displacement, catheter tract metastases, and tube blockage.[57] An ongoing trial, IPC PLUS, is looking at the efficacy of TPC placement versus TPC and talc pleurodesis, and may provide us with improved strategies for management of MPE.[58]

FUTURE DIRECTIONS

Although progress is being made for diagnosing MPM, treatment is not advancing so quickly. Identifying biomarkers for earlier disease detection, perfecting protocols for multimodal treatment, and developing novel therapeutic approaches are of utmost importance. Promising advancements in diagnostic tools, such as novel biomarkers in pleural effusions and serum, as well as IHC and signature miRNA, will help with earlier detection and accurate tumour profiling. Advancements in therapies, including immunotherapy, intrapleural gene therapy via adenovirus,[59] exosome-delivered miRNA, and other drug delivery systems,[60] as well as TPC with a drug-eluting coating to deliver intra-pleural sclerosing agents over time,[56] offer hope for a better diagnosis and treatment of this disease.

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UPCOMING EVENTS

2018 Progress and Controversies in Gynecologic Oncology Conference

19th–20th January 2018

Barcelona, Spain

The 2018 Progress and Controversies in Gynecologic Oncology Conference is an ideal opportunity for any healthcare professional involved in the diagnosis and management of gynaecological cancers to gain insight into the latest updates within the field. This interactive, 2-day event presents an opportunity for discussion with some of the world's leading experts in gynaecological malignancies, promoting better clinical outcomes for patients around the world.

Cancer Genetics Course 2018

29th January–1st February 2018

London, UK

Taught by members of the cancer genetics team at Guy's Hospital, London, UK, this skills-based course is a fantastic opportunity for healthcare professionals to improve their knowledge surrounding genetic counselling and the use of family history to better inform patients; sessions will also summarise the management strategies to be developed. Book your place now so you do not miss out on this fully accredited course.

33rd German Cancer Congress 2018 (DKK 2018)

21st–24th February 2018

Berlin, Germany

With the 33rd DKK congress approaching, this annual event is now recognised as the largest and oldest congress in German-speaking countries, focussing on cancer diagnostics and therapy. The 4-day event in early 2018 will bring together experts from the oncology field, as well as other relevant specialists within oncology patient care, to promote intense discussion and collaboration, benefitting the future of oncology medicine.

35th Annual Miami Breast Cancer Conference

8th–11th March 2018

Miami Beach, Florida, USA

The Miami Breast Cancer Conference 2018 will bring together medical, surgical, and radiation oncologists to discuss approaches to multidisciplinary care for breast cancer patients. The educational programme encourages all oncology specialists and healthcare professionals to attend the 4-day event and embark on a journey through the latest advances in oncology medicine, with a particular emphasis on applying these developments to clinical practice.

European Lung Cancer Conference 2018 (ELCC 2018)

11th–14th April 2018

Geneva, Switzerland

The ELCC 2018, organised by the European Society for Medical Oncology (ESMO) and the International Association for the Study of Lung Cancer (IASLC), provides an outstanding opportunity for collaboration, bringing together experts from around the world, with the aim of presenting the latest advances in the study of lung cancer. With a focus on lung cancer-related immunotherapy, screening, molecular backgrounds, and more, this event is one that will prove unmissable.

European Society for Therapeutic Radiology and Oncology Annual Meeting 2018 (ESTRO 37)

20th–24th April 2018

Barcelona, Spain

ESTRO 37 is the perfect chance for radiation oncology therapists to collaborate with numerous oncology organisations with the ultimate aim of improving cancer treatments. Healthcare and industry professionals will be in attendance, offering opportunities for delegates to enhance their professional development and network with peers and colleagues. Young scientists, who will form the future of oncology medicine, are also encouraged to attend.

25th Biennial Congress of the European Association for Cancer Research (EACR25)

30th June–3rd July 2018

Amsterdam, Netherlands

The EACR will be celebrating its 50th anniversary at its 25th Biennial Congress next year. The theme of EACR25 is 'from fundamental insight to rational cancer treatment', celebrating the journey of cancer treatments so far and promoting further advances within the field. Book your place at this diverse event now for a fantastic, unmissable 4 days, ideal for any oncology enthusiast wishing to spark their imagination and promote novel patient care.

European Society for Medical Oncology (ESMO) 2018 Congress

19th–22nd October 2018

Munich, Germany

We look forward to attending the ESMO 2018 Congress, as the organisers promise the presentation of continued, high-quality, innovative research and ideas that delve further into our increasing knowledge of cancer medicine. With a programme developed by >300 international experts, this 4-day event is the perfect opportunity to gain insight into the latest advances in the field, providing endless improvements for oncology patient care.

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