EMJ EUROPEAN MEDICAL JOURNAL

ONCOLOGY

ISSN 2053-4213 — Vol 7.1 • November 2019 • emjreviews.com

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"The peer-reviewed articles in this year's edition are as inspirational as ever."

Spencer Gore, CEO

+ ARTICLES

Editorial Board

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Aims and Scope

The European Medical Journal (EMJ) is an online only, peer-reviewed, open access general journal, targeted towards readers in the medical sciences. We aim to make all our articles accessible to readers from any medical discipline.

EMJ allows healthcare professionals to stay abreast of key advances and opinions across Europe.

EMJ aims to support healthcare professionals in continuously developing their knowledge, effectiveness, and productivity. The editorial policy is designed to encourage discussion among this peer group.

EMJ is published quarterly and comprises review articles, case reports, practice guides, theoretical discussions, and original research.

EMJ also publishes 16 therapeutic area journals, which provide concise coverage of salient developments at the leading European congresses. These are published annually, approximately 6 weeks after the relevant congress. Further details can be found on our website: www.europeanmedical-journal.com

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EMJ is supported by various levels of expertise:

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- Invited contributors are recognised authorities from their respective fields.
- Peer review, which is conducted by EMJ's Peer Review Panel as well as other experts appointed due to their knowledge of a specific topic.
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This Publication

European Medical Journal Oncology is published once a year. For subscription details please visit: www.europeanmedical-journal.com

Masthead

EMJ HEMATOLOGY 2019

EMJ Hematology 7.1 aims to provide you with a detailed review of the event, an account that is perfectly accompanied by a hand-picked selection of peer-reviewed articles.

VIEW ALL JOURNALS ←

Welcome

Valued readers, collaborators, and friends, I warmly welcome you to *EMJ Oncology 7.1*. Inside you will be treated to our independent review of this year's European Society for Medical Oncology (ESMO) congress as well as a notable selection of hand-picked peer-reviewed articles.

This year's ESMO meeting, held in sunny Barcelona, Spain, was the global stage for excellence in translational research where the congress revealed practice changing data and brought the attendees up to date with the hottest topics from across the oncology field. With a vast number of oncologists and attendees partaking in multidisciplinary debates across 5 days, we are certain that the congress will spur transformative therapies against cancer in the years to come.

For those of you that were unable to attend ESMO 2019, our abstract reviews present a collated array of the best abstracts from the speakers themselves. Furthermore, you will be able to read the latest news-updates in our congress review section, ranging from the use of liquid biopsy to identify complex lung cancer mutations to the potential of omitting postoperative radiotherapy in prostate cancer surgery. This edition also features an interview with the chair of ESMO's Young Oncologists Committee, addressing the need to continually improve career opportunities for the young and upcoming oncologists.

The peer-reviewed articles in this year's edition are as inspirational as ever. Dent et al. share their discovery of novel molecule for the targeting and downregulation of mutant RAS for the potential treatment of countless cancers. Targeting this mutated protein has been the focus of the oncology community for years, making this an exciting read for all. Also included is a fantastic review by Rygiel detailing the application of PARP inhibitors for patients with *BRCA*-mutated metastatic breast cancer. A comprehensive summary of PARP inhibitor clinical trials is given, as well as important aspects of efficacy, safety, and resistance. These are just two of a handful of peer-reviewed articles that we are sure you will enjoy.

With this being the 7th edition of *EMJ Oncology*, it has been encouraging to see the trailblazing advances that have been made in this ever-changing field. I am further inspired by the commitment and hard work of the entire EMJ team to ensure that with each year the journal material becomes more engaging. I would also like to extend my appreciation to everyone who contributed to *EMJ Oncology 7.1*, and I hope that everyone enjoys the incredible content that lies ahead as much as I did!

Spencer Gore

Chief Executive Officer, European Medical Group

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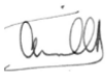
Foreword

This edition of *EMJ Oncology 7.1* offers to the readers a wide range of topics and papers in relation to cancer diagnosis and management, as well as a comprehensive review of the European Society of Medicinal Oncology (ESMO) 2019 congress.

Branchial cleft cyst (benign lesion) and accessory breast cancer are anecdotal clinical situations that merit optimal diagnosis in order to offer the patient the best therapeutic approach. These topics are given some spotlight in case reports by Akheel and Moustafa et al., respectively. Also included within is another case report by Palaniappan et al. depicting epithelial myoepithelial carcinoma, a very rare histology of the salivary gland and a difficult tumour to diagnose. A future area of interest will be to depict the molecular features of this tumour using new molecular technologies in order to understand the molecular biology and the carcinogenesis process of this malignancy.

An additional paper discusses PARP inhibitors, which are a good therapeutic option for germline *BRCA*-mutated breast, ovarian, and prostate cancers. Two major research questions are raised to which work still needs to be done: why do some *BRCA* wild-type tumours respond to this therapy, and conversely, why do some germline *BRCA*-mutated tumours not respond?

Rygiel provides a very interesting paper summarising the introduction of checkpoint inhibitors in the management of triple negative breast cancer, a poorly treated malignancy that requires increased attention from the research community. Finally, Neratinib, an irreversible pan HER inhibitor, might have indirect effects on mutated RAS that could have widespread implications for a number of different cancers.



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Congress Review

Review of the European Society for Medical Oncology (ESMO) Congress 2019

Location: Fira Barcelona Gran Via – Barcelona, Spain

Date: 27.10.19–01.10.19

Citation: EMJ Oncol. 2019;7[1]:10-23. Congress Review

Barcelona, a city with undisputed character, ambience, and beauty, played host this year to the prestigious annual European Society for Medical Oncology (ESMO) Congress 2019 for which we are happy to provide a comprehensive review, leaving nothing to be desired. The meeting welcomed 28,571 participants from 138 countries to join the discussion about the future of oncology with a focus on translating science into better patient care.

The congress was held in the hometown of ESMO president Dr Josep Tabernero, who in his second year of presidency opened the ceremony with a speech reflecting on the thriving community of ESMO. With 23,000 members and continuous expansion, the heavily influential meeting harnesses the power to change the way healthcare professionals apply their expertise in clinical practice. Dr Tabernero emphasised that the 360° approach exhibited by the society begins with "rigorous science"; this is a society which acts and helps to evolve the face of oncology. ESMO is a significant global platform at the forefront of oncology where ground-breaking discovery and science prevails. This year's meeting was not unlike previous years, and powerful conversations ensued.

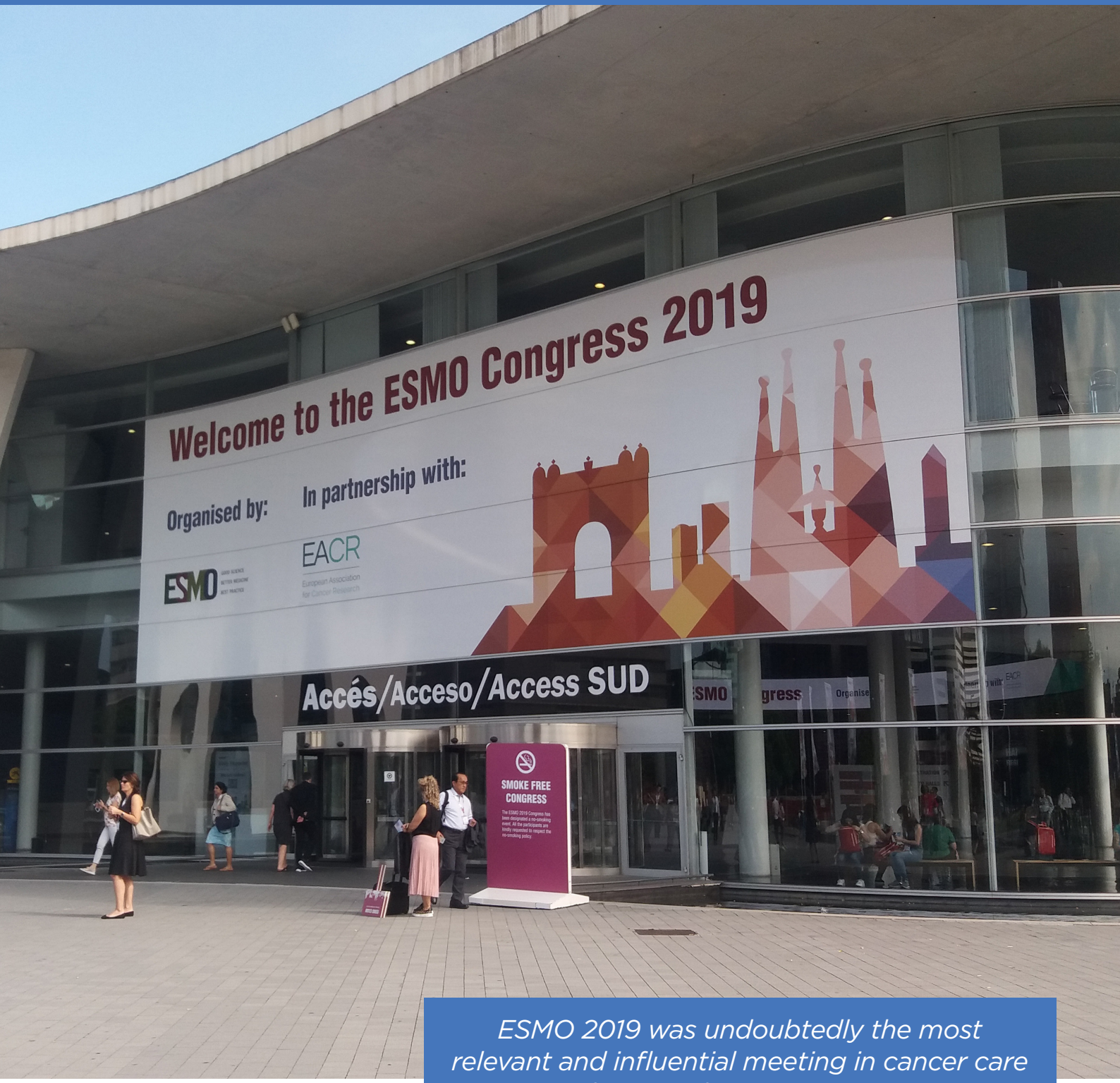
ESMO 2019 received a record-breaking 3,904 late-breaking abstracts, 1,736 of which were presented to delegates who attended the meeting. Herein, we have included a hand-picked selection of abstracts summarised by lead authors who presented their research at the congress. The abstracts summaries included in this review are explorations of some of the hottest topics in the field of oncology, including: treatment of non-resectable or metastatic soft tissue sarcomas by pazopanib in patients who are not eligible for chemotherapy; the burden of cancer in Europe; and results from a descriptive real-life study of collaborative management of immune-related adverse events induced by immune checkpoint inhibitors, amongst many more. The arsenal of outstanding abstracts presented at this year's ESMO Congress are respectfully represented in our collection of summaries.

Numerous sessions presented at the Congress provided exciting updates about cutting-edge research in the field. The studies presented gave plenty to consider, revolutionising conventional practices within the field. The RADICAL-RT trial addressed the side effects of postoperative radiotherapy in prostate cancer versus the benefits in an investigation into whether radiotherapy is required in these circumstances. Results from the ClarIDHy Phase III trial presented at Congress were the first to show the clinical benefit of targeted therapy for the treatment of cholangiocarcinoma and is an important study in support of tumour mutation profiling. Additionally, the combination of chemotherapy and immunotherapy is a prospect seen with utmost gravity for the treatment of bladder cancer, and is presented in the coming pages.

The session 'Level playing field: working for gender balance in oncology', which began with the striking statement that one out of six presidents in oncological societies are woman, is presented as a feature in this review. This was a stimulating discussion about the need for female representation in oncology at a global level, which tackled the balance of gender in medicine and argued that concerns are more far-reaching than a singular closure of the economic gap. The evolutionary advancements of the gender gap in oncology has a long way to go; further progression is mandatory at a faster pace, and necessary actions to take were also highlighted to build on plans for a gender-balanced future.

We had the pleasure of conducting a featured Congress interview with Guillem Argilés, Chair of the Young Oncologists Committee in ESMO.

"ESMO is a significant global platform at the forefront of oncology where ground-breaking discovery and science prevails."



ESMO 2019 was undoubtedly the most relevant and influential meeting in cancer care to happen this year in Europe

Dr Argilés reflects on his week at the congress, shares his thoughts about the benefits conferred by having a mentor, the effect this may have on young oncologists, and what young oncologists can teach the more experienced members of the ever-growing oncology committee.

ESMO 2019 was undoubtedly the most relevant and influential meeting in cancer care to happen this year in Europe, and provided an important platform for breakthrough science and discovery in the field. The initiatives taken at ESMO 2019 will continue to build throughout the next 12 months, and the discussion will inevitably recommence next year at the ESMO 2020 congress in Madrid. To devour more congress content, please enjoy the following review of ESMO's 2019 annual meeting.

Liquid Biopsy Successfully Used to Identify Complex Mutations in Lung Cancer Mutations

TARGETED medicine approaches may be advisable for non-small cell lung cancer (NSCLC) patients based on a simple blood test as opposed to more invasive tumour biopsies. This message was delivered as part of a press release on 30th September 2019 at the ESMO 2019 Congress in Barcelona, Spain. These liquid biopsies are able to detect circulating tumour DNA fragments harbouring NSCLC-specific genomic aberrations such as *ALK* gene rearrangements, and these results could hold wider implications for the ways in which clinicians employ these tests for a variety of other cancer screens.

The Phase III BFAST trial incorporated ≥2,000 untreated NSCLC patients into next-generation sequencing analysis involving blood tests for numerous driver genetic mutations. Of the cohort, 119 patients (5.4%) were seen to have ALK+ disease, and in a subsequent 12-month follow-up in which ALK-targeting alectinib was prescribed to 87 of these patients, 75.9% demonstrated a durative response over the 12 months. Although median progression-free survival was not reached, 12-month progression free survival reported by investigators was 78.4%.

The study represents a breakthrough in NSCLC diagnosis; despite our enhanced ability to identify targetable genetic mutations, challenges existed regarding the suitability of tumour samples for analysis. *ALK* gene rearrangements, although often actionable, are hard to detect, making the possibility of using blood screening for their identification all the more attractive.

"It is encouraging to see that increasing numbers of patients with lung cancer can benefit from liquid biopsy to identify their disease mutation instead of tissue samples," said Prof Alberto Bardelli, University of Turin, Turin, Italy, commenting on the study. "At present the technology is quite expensive but as it becomes more widely used, the cost is likely to come down so that testing becomes more affordable and available in daily practice."

"It is encouraging to see that increasing numbers of patients with lung cancer can benefit from liquid biopsy to identify their disease mutation instead of tissue samples,"

Combination Immunotherapy Significantly Improves 5-Year Metastatic Melanoma Survival

PREVIOUSLY regarded as untreatable due to factors such as chemotherapy ineffectiveness and the treatment difficulty following its spread, metastatic melanoma has been dealt a significant blow as seen from the CheckMate 067 trial results. In this study, one in two metastatic melanoma patients were seen to survive following 5 years of combination immunotherapy, a significant improvement from the standard-of-care. The results were presented as part of a press release on the 28th September 2019 at the ESMO Congress in Barcelona, Spain.

In the longest Phase III follow-up for a checkpoint inhibitor combination therapy, CheckMate 067 enrolled 945 patients with previously untreated Stage III or IV melanoma who were randomly allocated in a 1:1:1 ratio to nivolumab plus ipilimumab, nivolumab plus placebo, or ipilimumab plus placebo. Ipilimumab monotherapy was also compared to each of the nivolumab arms.

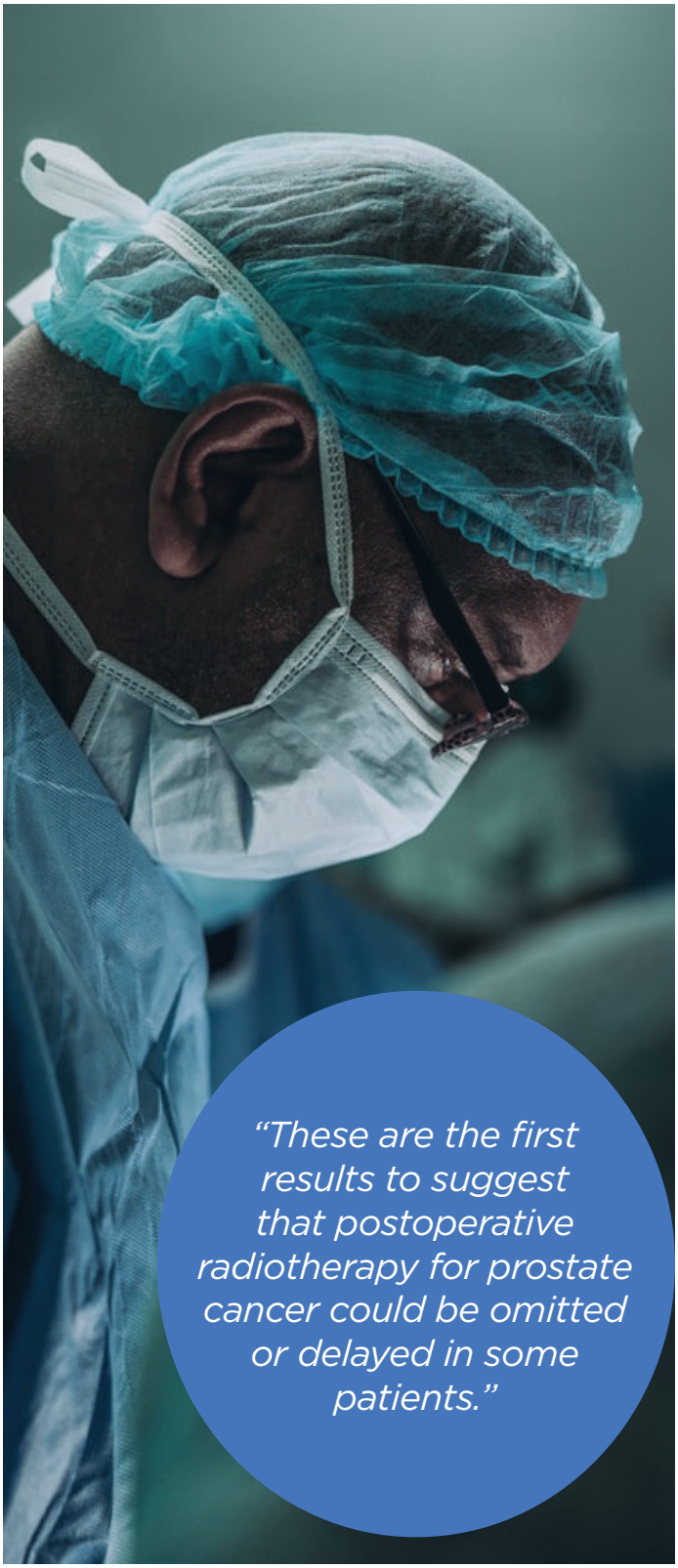
Representing a major improvement on what had be seen historically, 5-year overall survival rates for the nivolumab plus ipilimumab, nivolumab only, and ipilimumab only arms were 52%, 44%, and 26%, respectively. The proportion of patients currently alive and free from subsequent therapy was 45% and 58% for ipilimumab and nivolumab respectively; however, combination therapy increased this to 74%.

Prof James Larkin, study author from Royal Marsden NHS Foundation Trust, London, UK, commented that "this treatment transforms the disease to one with an approximately 50% cure rate. The priority now is to find ways to cure the remaining 50%" Despite this success, the researchers state that there is still work needing to be done in determining which patients are most likely to benefit from combination immunotherapy. "The decision on which treatments to give is a matter for doctors to discuss with individual patients and their families." Additional research is also required for identifying patients resistant to immunotherapy, a vulnerable demographic requiring a different therapeutic approach.

"this treatment transforms the disease to one with an approximately 50% cure rate. The priority now is to find ways to cure the remaining 50%"

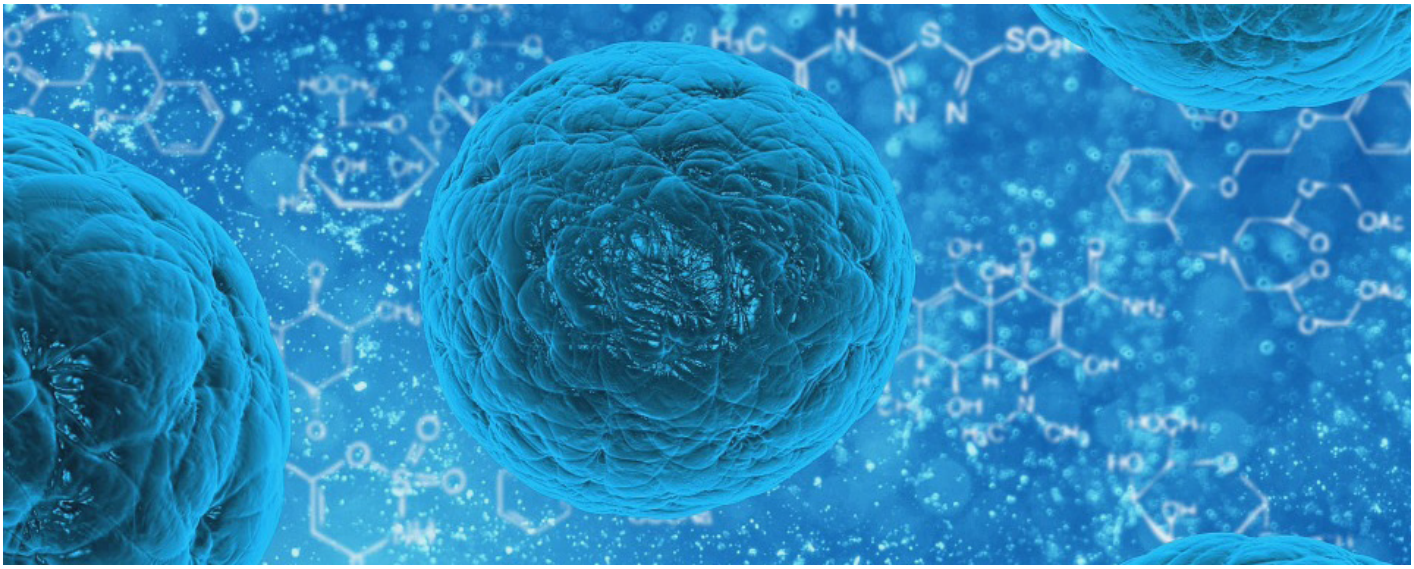
Postoperative Radiotherapy No Longer Necessary in Prostate Cancer Surgery

MEN with prostate cancer may not be required to undergo postoperative radiotherapy for prostate cancer according to the results of a recent study presented at this year's ESMO Congress in Barcelona, Spain, and reported in a press release dated 27th September 2019. Speculation surrounding the detrimental side effects versus the benefits of radiotherapy following prostate cancer surgery was addressed in the RADICALS-RT trial, the largest trial of its kind.



The first author of the study Prof Chris Parker, Royal Marsden NHS Foundation Trust and Institute of Cancer Research, London, UK, commented: "The results suggest that radiotherapy is equally effective whether it is given to all men shortly after surgery or given later to those men with recurrent disease. There is a strong case now that observation should be the standard approach after surgery and radiotherapy should only be used if the cancer comes back." This suggests that side-effects, which include urethral stricture and urinary incontinence and that are experienced by many men following radiotherapy, could be avoided. The authors note that whilst complications after surgery alone are still a risk, surgery plus radiotherapy confers an even greater risk.

The study was presented as part of a meta-analysis with results from the RAVES and GETUG-AFU17 trials, a collection of data derived from a total of 2,151 men. The results of the meta-analysis were said to have provided further evidence towards the observational and salvage radiotherapy approach to treatment. Dr Xavier Maldonado, Hospital Universitari Vall d'Hebron, Barcelona, Spain, confirmed, "These are the first results to suggest that postoperative radiotherapy for prostate cancer could be omitted or delayed in some patients." He went on to say that monitoring would be paramount if patients required salvage radiotherapy and longer follow-up to reach the primary endpoint of the trial: to ensure full toxicity report and freedom from distant metastases at 10 years.



Promising Response for First-Line Immunotherapy in Advanced Hepatocellular Carcinoma

DATA on first-line immunotherapy for the treatment of advanced hepatocellular carcinoma (HCC) may indicate preferable clinical outcomes compared to current standard of care for this type of cancer. These results were from a recent study presented at this year's ESMO Congress in Barcelona, Spain, and reported in a press release dated 27th September 2019.

According to the study, first-line nivolumab showed improvements in overall survival, response rate, and safety profile compared to sorafenib which is used as the current treatment for advanced HCC. HCC is typically diagnosed in later stages of the disease, by which point therapeutic options are not readily available. The study author Dr Thomas Yau, University of Hong Kong, Shatin, China, commented on the importance of the results: "The encouraging efficacy and favourable safety profile seen with nivolumab demonstrates the potential benefit of immunotherapy as a first-line treatment for patients with this aggressive cancer."

"...it is becoming apparent that immunotherapy could have a role for the first-line treatment of advanced HCC and the differences in response rates are clinically meaningful."

The trial included 743 participants with advanced HCC who were randomised to receive either nivolumab or sorafenib. Participants who took nivolumab showed a greater median overall survival of 16.4 months compared to those who took sorafenib, who had a median overall survival of 14.7 months. The data from the study did not achieve its statistically significant prespecified primary endpoint for overall survival, therefore the data must be considered accordingly; however, the study did show increased overall survival, higher complete response rate, and participant-reported improved quality of life with nivolumab, suggesting that clinical benefit was observed. Although the study data did not meet its predefined threshold, it offers important insights into HCC therapy, as highlighted by Dr Angela Lamarca, Christie NHS Foundation, Manchester, UK: "...it is becoming apparent that immunotherapy could have a role for the first-line treatment of advanced HCC and the differences in response rates are clinically meaningful."

Increased Anticancer Drug Costs: Are They Worth it?

THE COST of treatment is a major reason for patients to be denied access to newer anticancer treatment drugs. According to two studies presented at a press conference on 27th September 2019 at the annual ESMO congress, Barcelona, Spain, many novel anticancer medicines provide little value for patients compared to standard treatment.

The two studies investigated the connection between clinical benefit and pricing in Europe and the USA in regard to novel cancer medicine. Medicines introduced in the last 15–20 years for solid tumours were investigated to determine whether their monthly treatment costs were associated with clinical benefits. The focus was to determine improved outcomes for factors such survival, quality of life, and treatment complications compared to the standard treatment options.

The first study's results revealed that almost half of the new drugs for treatment of solid tumours approved in Europe between 2004 and 2017 had low added value scores on the ESMO Magnitude of Clinical Benefit Scale (ESMO-MCBS). Furthermore, more than two-thirds had low value on the Added Therapeutic Benefit Ranking (ASMR) scale used by French drug regulators. Dr Marc Rodwin, Law School, Suffolk University, Boston, USA, stated that "most of the new drugs had low added value, so doctors and patients shouldn't assume that just because a drug is new, it's going to be better." Data revealed that on average new drug costs were €2,525 more per month than active control comparator drugs for the same cancer type.

"most of the new drugs had low added value, so doctors and patients shouldn't assume that just because a drug is new, it's going to be better."

Drugs approved for adult solid tumours in four European countries and in the USA from 2009–2017, displayed no link between drug cost and clinical benefit measures by ESMO-MCBS and the American Society of Clinical Oncology Value Framework (ASCO-VF) in the second study. However, median drug prices in Europe were less than half of the USA prices, and the average monthly drug cost for drugs with low benefit scores on ESMO-MCBS ranged from €3,944–4,770 ($4,361–5,273) in the European countries compared to €11,249 ($12,436) in the USA.

These studies delineated that drug costs were not associated with clinical benefit score in the countries investigated. Co-author Prof Kerstin Vokinger, University of Zurich, Switzerland, stated that "some of the more expensive drugs for prostate and lung cancer in Switzerland had lower ESMO-MCBS scores, while cheaper drugs had higher scores. Therefore, it is important that drug pricing is aligned with clinical value and that resources are spent on innovative medicines that offer improved outcomes."

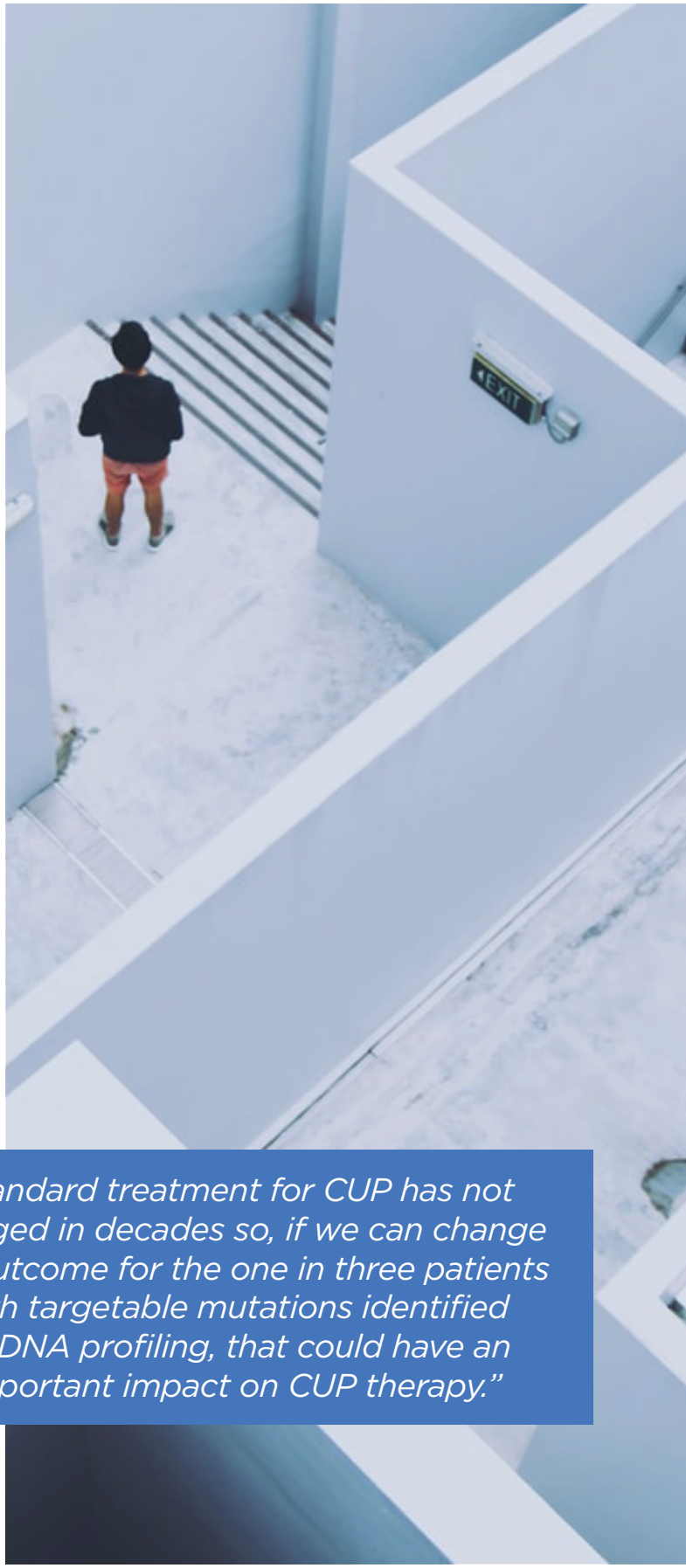
Drugs Targeted to DNA Alterations may Improve Patient Outcomes for Cancers of Unknown Primary

METASTASISED tumours are difficult to treat for many reasons, one of which being that no primary tumour site can be identified by the time of diagnosis. This results in approximately one in three patients not being adequately treated with standard chemotherapy. Novel treatments for these cancers, commonly known as carcinoma of unknown primary (CUP), are now being revealed by DNA profiling, as revealed in a study at ESMO 2019 and stated in a press release dated the 28th September.

Following an unsuccessful identification of the cancer site of origin from which the cancer has spread, the patient receives standard anticancer treatment, with little chance of a cure or palliative care to relieve symptoms. Surprisingly, CUP affects 1 in 15 cancer patients, and of these, only 1 in 10 survive for 1 year. Results from a study presented at ESMO 2019, that analysed 303 CUP tissue samples in search of DNA changes that could be targeted, revealed that 32% of the cancers could have been targeted with recent, mutation-specific drugs.

The first author of the study, Prof Jeffrey Ross, Upstate Medical University, Syracuse, New York, USA, commented that: "standard treatment for CUP has not changed in decades so, if we can change the outcome for the one in three patients with targetable mutations identified by DNA profiling, that could have an important impact on CUP therapy."

The techniques that were used in this study are now being applied in the ongoing prospective CUPISCO trial, which is randomising CUP patients to either standard platinum-based chemotherapy or individualised targeted treatment or immunotherapy based on their tumour's genetic mutations. Prof Ross urged that: "CUP is a bit of a pariah because people don't understand it and assume that nothing can be done. We need to change that attitude and encourage clinicians to look for and treat the drivers of each patient's disease as shown by DNA profiling."



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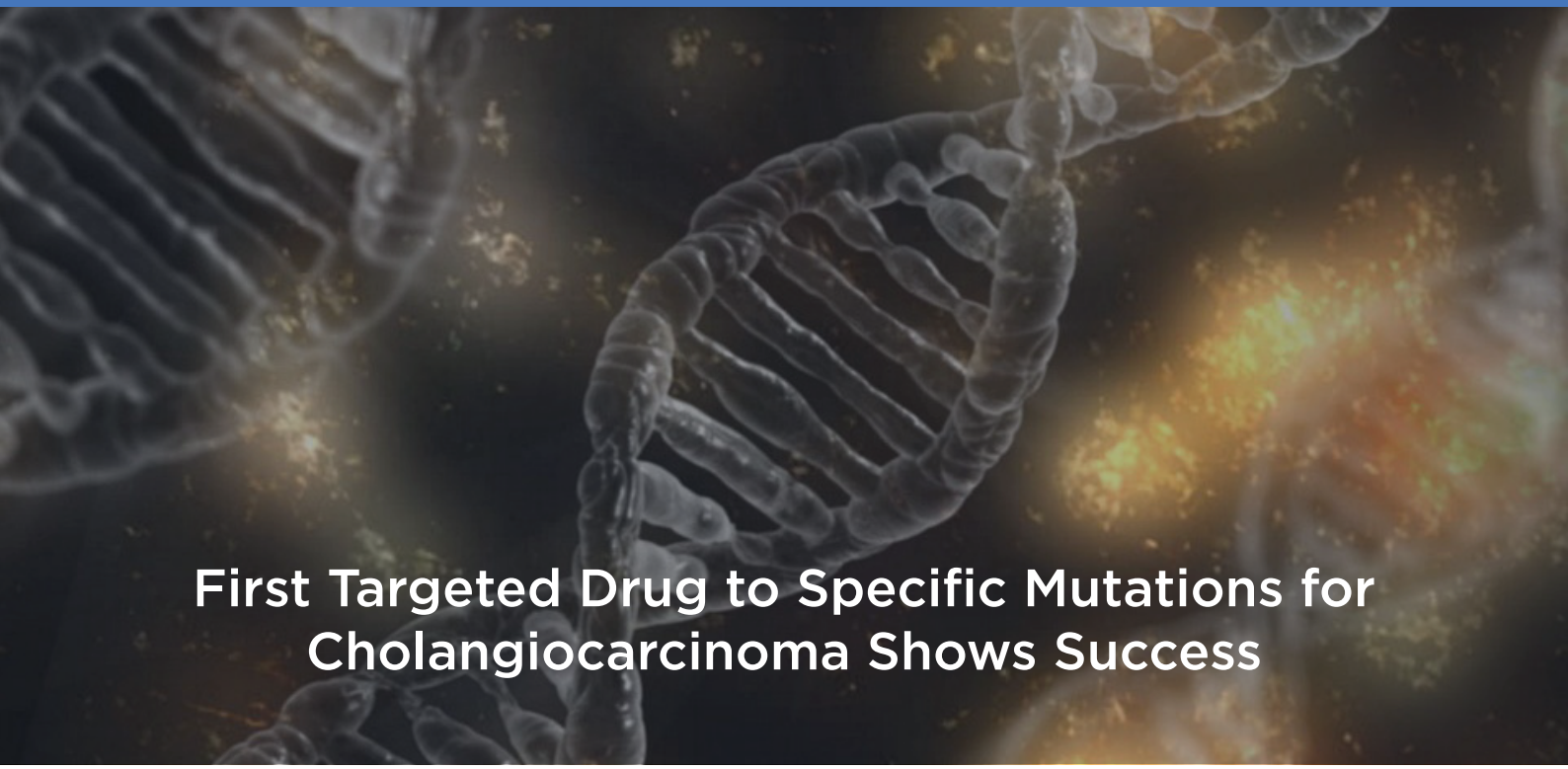


First Targeted Drug to Specific Mutations for Cholangiocarcinoma Shows Success

CHOLANGIOCARCINOMA is an aggressive subtype of bile duct cancer and has a poor prognosis. Most patients die from the disease; therefore, new treatments that are more directed to the specific disease need to be developed. The results of the ClarIDHy Phase III trial, that were presented at the ESMO 2019 Congress in a press release dated 30th September, are the first to show the clinical benefit of targeted therapy for the treatment of cholangiocarcinoma.

Dr Chris Verslype, University Hospital Leuven, Leuven, Belgium, noted: "It is the first time in cholangiocarcinoma that a Phase III study tests a drug targeted to a specific anomaly, and it seems to work. Importantly, you identify suitable patients by selecting them for *IDH1* mutation. It is precision medicine brought to the clinic. And it is very likely to change clinical practice. It will, for sure, drive the further development of targeted therapy for this disease."

The study investigated whether the drug ivosidenib, that targets the isocitrate dehydrogenase 1 (I*DH1*) mutation that is seen in 15% of patients, improves the progression-free survival (PFS) in cholangiocarcinoma patients. Patients with advanced cholangiocarcinoma and *IDH1* mutations (N=185) were randomised to ivosidenib or matched placebo. When the patient's disease progressed, they could crossover from placebo to ivosidenib.

"It is the first time in cholangiocarcinoma that a Phase III study tests a drug targeted to a specific anomaly, and it seems to work."

Median PFS was significantly longer in the ivosidenib group compared to placebo (2.7 months versus 1.4 months, respectively; hazard ratio: 0.37; 95% confidence interval: 0.25–0.54; p<0.001). Furthermore, the median PFS rate at 6 months for the ivosidenib group was 32% compared to no patients being progression free at this timepoint.

"The findings mean all patients with cholangiocarcinoma should be tested for *IDH1* mutation. Tumour mutation profiling should be a new standard for the care for patients with this heterogeneous tumour type," commented the study author Dr Ghassan Abou-Alfa, Memorial Sloan-Kettering Cancer Center, New York City, New York, USA.

Novel Front-Line Treatment Changes Treatment Outlook for Non-Small Cell Lung Cancer

OSIMERTINIB significantly lengthens overall survival in patients with the *EGFR* exon 19 L858R mutation implicated in advanced non-small cell lung cancer (NSCLC), compared to older generation EGFR-tyrosine kinase inhibitors (*EGFR*-TKI). These results from the FLAURA trial were presented at a press release on 28th September 2019 at the ESMO congress in Barcelona, Spain.

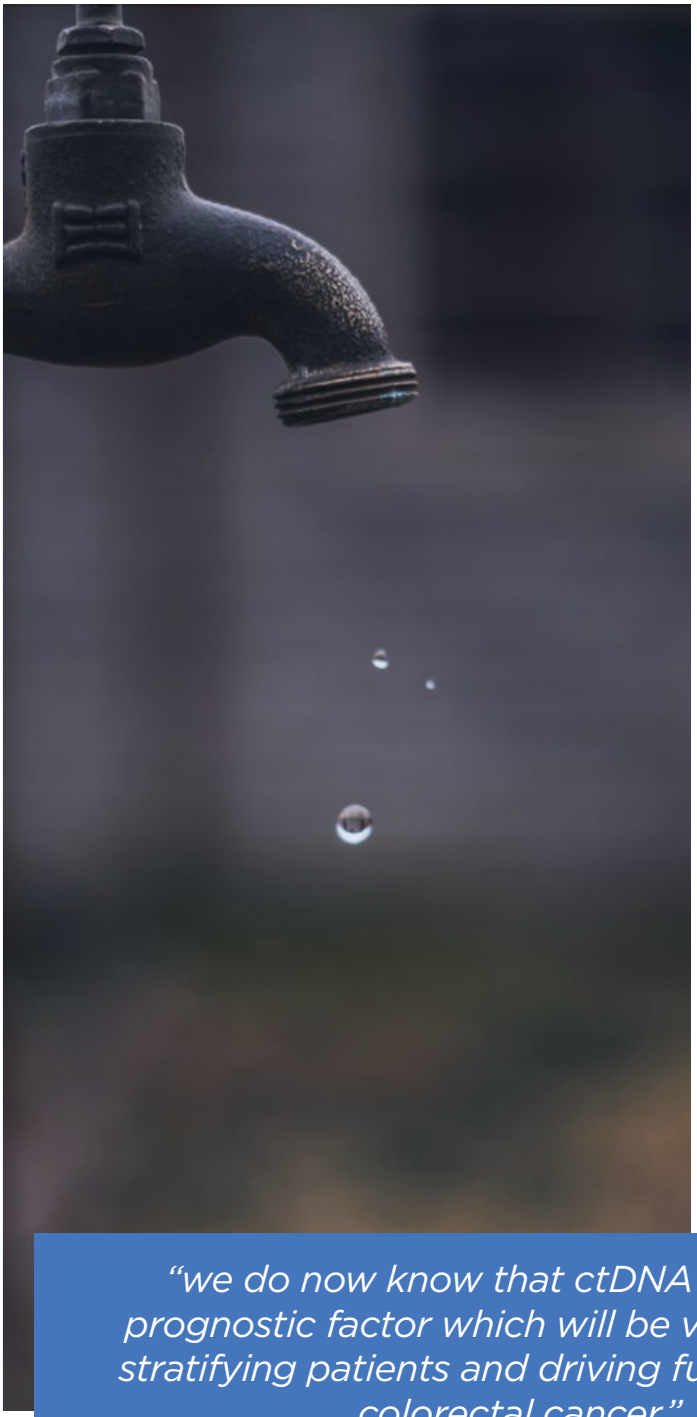
Results from the trial show that median survival with osimertinib was 38.6 months compared to 31.8 months with first generation *EGFR*-TKI, with a hazard ratio of 0.799 (p=0.0462). Furthermore, results revealed that 54% of patients in the osimertinib group were alive at 3 years compared to 44% in the standard care group. Prof Suresh Ramalingam, Winship Cancer Institute of Emory University, Atlanta, USA, stated that "the survival results are both statistically significant and clinically meaningful with first-line osimertinib for *EGFR* mutated patients. This is the first time a TKI has proven to extend survival relative to another TKI in lung cancer therapy."

Prof Ramalingam also stated that 31% of the patients in the control group crossed over to the osimertinib arm after disease progression; as such, a total of 47% of patients in the control group received post-study therapy. Prof Ramalingam validated the findings and stated that this was "consistent with what we would expect in the real-world setting, since only about 50% of patients develop the *T790M* mutation and will be candidates for osimertinib."

According to Prof Ramalingam "FLAURA met both its primary and key secondary endpoints and showed a favourable safety profile for osimertinib. The results further reinforce the clinical utility and superiority of osimertinib in the front-line setting. Based on these data, osimertinib should be the preferred front-line therapy for *EGFR*-mutated lung cancer patients. "Dr Pilar Garrido, Ramón y Cajal University Hospital, Madrid, Spain added that the results are positive for patients and also important for the debate about the best treatment sequence considering that osimertinib is the only TKI approved for second-line treatment in patients who develop resistance to *T790M*. She additionally added that it is important that patients are informed about the survival advantage, yet should the treatment fail the only option is chemotherapy.

"This is the first time a TKI has proven to extend survival relative to another TKI in lung cancer therapy"

Liquid Biopsy Could Play Important Role in Colorectal Cancer Diagnosis



"we do now know that ctDNA is a major prognostic factor which will be very useful in stratifying patients and driving future trials of colorectal cancer,"

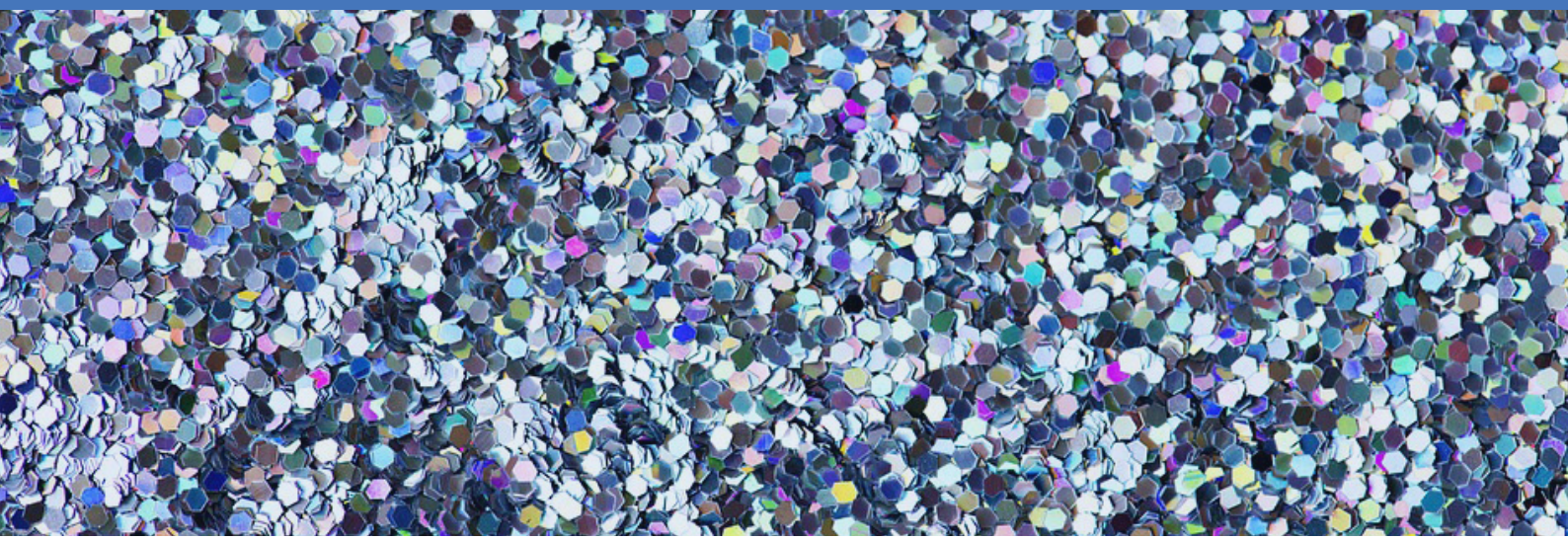
LIQUID biopsy may be of increasing importance in the identification of colorectal cancer (CRC) in patients who are likely to experience a relapse following surgery, and may also allow the opportunity to optimise treatment for patients on an individual basis, according to research presented at ESMO Congress 2019, Barcelona, Spain, and in a press release dated 28th September 2019.

The Phase III IDEA-FRANCE trial studied 805 patients who underwent liquid biopsy before having chemotherapy to treat Stage III CRC. Of the cohort, 109 patients had circulating tumour DNA (ctDNA) found in their blood. Within this group, 2-year disease free survival (DFS) was found to be 64%, compared with 82% of the ctDNA negative group.

Study author Prof Julien Taieb, Prof Hôpital European Georges Pompidou, Paris, France, discussed the study: "In this large prospective trial, we confirmed that ctDNA is an independent prognostic factor in colorectal cancer and that approximately six out of ten patients who are ctDNA positive will remain disease-free 2 years after standard adjuvant chemotherapy, compared to eight out of ten of those who are ctDNA negative."

Results from the study also showed that adjuvant treatment over 6 months was superior to 3 months of treatment, in both ctDNA positive and ctDNA negative patients. Treatment for 6 months in ctDNA positive patients was also found to result in a similar prognosis to ctDNA negative patients who underwent 3 months of treatment. In 90% of cases, adjuvant therapy was folinic acid, fluorouracil, and oxaliplatin.

"ctDNA testing did not predict which patients should have 3 or 6 months of adjuvant chemotherapy and there is continuing debate over the optimal type and duration of treatment for patients who are ctDNA positive, but we do now know that ctDNA is a major prognostic factor which will be very useful in stratifying patients and driving future trials of colorectal cancer," continued Prof Taieb, adding: "In all subgroups, ctDNA positive patients who only had 3 months of adjuvant therapy had the worst prognosis."



Exciting New Prospect for Bladder Cancer Patients

> *"This is remarkable. We are now eager to see if patients receiving the two therapies together live longer, and with a similar quality of life, than those receiving chemotherapy and immunotherapy alone or sequentially."*

COMBINATION therapy with chemotherapy and immunotherapy could offer improved outcomes for some bladder cancer patients according to the results of a recent study presented as part of an ESMO press release dated 30th September. The study compared the combination therapy to chemotherapy or immunotherapy alone of sequentially to assess whether progression-free survival could be improved.

Currently, cisplatin-based chemotherapy is the first-line treatment for patients with metastatic urothelial cancer, and immunotherapies such as the PD-L1 inhibitors atezolizumab and pembrolizumab are approved for patients ineligible or unresponsive to chemotherapy. The current study, named IMvigor130, is the first to assess the outcomes for patients administered a combination of both treatments, whether they are eligible or ineligible for chemotherapy. The study enrolled 1,213 patients with metastatic urothelial cancer from 35 countries and randomised them 1:1:1 to receive A) atezolizumab plus platinum-based chemotherapy, B) atezolizumab alone, or C) placebo plus platinum-based chemotherapy. Chemotherapy plus atezolizumab improved the median time to progression of metastatic tumours by 2 months in comparison to chemotherapy alone. Furthermore, the patients in Arm A had an 18% reduced likelihood of progression. An additional trend was noted for improved survival in patients with overexpression of PD-L1 who were treated with atezolizumab alone compared to chemotherapy.

Dr Enrique Grande, MD Anderson Cancer Centre, Madrid, Spain, was lead author of the study and commented: "This is a new option for the upfront treatment of patients with metastatic urothelial cancer. Longer follow-up is needed on overall survival and we will continue to search for biomarkers to identify which patients respond best to this therapy." Dr Ignacio Durán, Hospital Universitario Marques de Valdecilla-IDIVAL, Santander, Spain, added: "This is remarkable. We are now eager to see if patients receiving the two therapies together live longer, and with a similar quality of life, than those receiving chemotherapy and immunotherapy alone or sequentially. The interim analysis of overall survival seems to be promising, but data are immature: overall survival data are needed to consider the combination of chemotherapy and immunotherapy as a new standard of care."

Balancing the Scales: The Fight for Female Representation in Oncology

Michael Dodsworth
Editorial Administrator

As exciting and momentous as the progression in medical oncology continues to be, a different type of progression is still sorely needed across the field and wider healthcare landscape. Few could argue over the importance of gender parity across different industries in today's society; however, the debate should perhaps not be focussed on the validity of the argument, but rather on the specific areas needing attention and the stepwise approaches employed to meet them. We are in the midst of a wave of proactive action being taken to increase female representation in the sciences, and ESMO are putting their best foot forward to face this challenge.

Taking its first breath in 2013, the ESMO Women for Oncology Committee was formed with the central idea of advancing the careers of female oncologists and helping them to become the leaders of tomorrow. This mission is rooted in concepts such as highlighting female leaders seen as models of excellence and serving as a platform to connect and endorse relevant initiatives. "The ESMO Women for Oncology Committee is a team of professionals, distinguished by their commitment to generating awareness and promoting equal career-development opportunities for female oncologists," proclaims the Chair of the committee and ESMO president elect, Dr Solange Peters. "Offering the same opportunities for success to every professional, irrespective of gender, race or age, leads to a merit-based system that can advance research and practice in order to provide optimal care for our patients."

A joint session organised by the Women for Oncology Committee and Young Oncologists Committee titled 'Level Playing Field: Working for Gender Balance in Oncology' delved deeper into the topic at the 2019 ESMO congress. Dr Peters opened the session with an alarming statistic: only one sixth of the major oncology societies' presidents are woman. Considering the influence that accompanies such a position, especially pertaining to policy change, the message was clear: progression is not happening quickly enough. Dr Peters provided weight to this point through acknowledgement of the World Economic Forum Global Gender Gap 2018 report, which uses four sub-indexes to assess the gap between men and women: 1) economic participation and opportunity; 2) educational attainment; 3) health and survival; and 4) political empowerment.[1] Whilst markers two and three have improved considerably over the years, a stark estimate was delivered over the

time it will take to attain truly equal economic participation and political empowerment: at the current rate of progression, it would take 202 years to reach this milestone. What followed throughout this session, however, were obvious signs of optimism to how this challenge is being faced.

Carrying on from this introduction, Dr Sabine Oertelt-Prigione, Strategic Chair for Gender in Primary and Transmural Care, Radboud University, Nijmegen, the Netherlands, enlightened the audience on the "struggles, joys, and lessons learned from an atypical career." Whilst acknowledging the challenges she had faced during her career because of her sex, age, and parental obligations, Dr Oertelt-Prigione nevertheless highlighted her being Caucasian, cis-gendered, and able-bodied, noting the importance of giving a platform to all minorities in order to inspire real change. Contextualising these differences is also important: "We are all the products of our life experiences. We have perspectives based on what we see and do [...] and the things we are confronted with every day. All of our experiences are somewhat individualised, and we need to take that into consideration: that's empowerment."

Dr Oertelt-Prigione proceeded to identify key messages she had learnt throughout her international career, including the importance of knowing one self's aspirations and values, the building of strong support networks, the realisation that a role should fit the individual (and not the other way round), and thoughts towards role models: it is of course ideal to identify with minorities in positions of power, however, if these role models are not present in your field, it can be valuable to realise that there may be no perfect role model, and that instead, it is important for the individual to rise and become their own.

Offering a far different perspective, Ms Michelle McIssac, Economist for the Health Workforce Department of the World Health Organization (WHO), presented statistics generated across two separate reports regarding female representation in healthcare. Women represent 70% of the global healthcare workforce, and contribute an estimated $3 trillion to the global healthcare economy annually.[2] The WHO have identified four areas to dedicate attention to in order to better represent and empower this significant arm of the healthcare community: occupational segregation, 'decency' of work, leadership and government, and the gender pay gap. As well as through engaging in conversation with global policy makers, the WHO have incorporated gender into their operational programming and performance evaluation, including a recent proposal to consider factors such as impactful integration of gender equity and rights for self-appraisal. Partner organisations and individuals can also join the conversation through a world-wide health force network co-chaired by the WHO called the 'gender equity hub',[3] aimed at addressing gender inequalities and amplifying initiatives to the global level.

Dr Guillem Argilés, Chair of the Young Oncologists Committee, concluded the presentation segment of the session by highlighting the commitment of this arm of ESMO towards collaborating with their colleagues from the Women for Oncology Committee to disseminate key incentives throughout the society. This sort of collaboration is surely reflective of the situation across global healthcare networks, in which a unified approach to gender representation is needed. Part of the service that these sessions provide is to act as a platform for the sharing of ideas regarding the introduction of gender balance into the workplace and on how to overcome everyday hurdles. The latter half of the session allowed the audience to get involved in the debate, discussing important issues, such as the steps that can be taken at the local level to promote gender equality, how to find a good mentor, how to manage time effectively (i.e., clinic and family), and the fostering of individual resilience to minimise the risk of burnout. Such practical and implementable lessons can allow young female oncologists to improve their working lives immeasurably, and when considered collectively, lead to far greater representation and empowerment of this demographic.

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Congress Interview

Prof Dr Guillem Argilés

Clinical Investigator
Vall d'Hebron Institute of Oncology (VHIO), Vall d'Hebron University Hospital, Barcelona, Spain

European Society for Medical Oncology (ESMO) Young Oncologists Committee

Q1 Can you please describe the role of the Young Oncologists Committee in ESMO, and what your personal duties are?

The Young Oncologists Committee is in charge of conveying the needs of the young oncologists across Europe within the society, and trying to work together with other ESMO committees to create policies and concrete actions focussed on improving the career opportunities and training opportunities of the youngsters.

Nearly 40% of the ESMO members are under the age of 40, which is the threshold for considering 'young' or 'senior' oncologists. Because of the fact that a significant proportion of ESMO members are young, there is ample reason to have a designated committee whose aim is to represent those interests. The committee was founded in 2001, meaning it has been an important part of my entire working career.

Q2 Did you have mentors, either personally or professionally, that helped you on your oncology journey, and if so, is this something that other young oncologists could benefit from?

Of course. I have one mentor right now, Josep Tabernero, who is the current president of ESMO. He has been my mentor for the last 10 years, and before him I had another. Having a mentor is very important in the medical oncology panorama, especially if you want to develop a relevant career; what I mean by relevant is to have an international career outlook. This field can be a very difficult one to progress in, not only because it's competitive, but also because you yourself need to try to acquire the necessary skills to become a good physician whilst at the same time start developing a scientific profile within the publication landscape. It's also important to develop one's visibility and communication skills. Thus, with so many important things to keep in mind throughout a medical career it is essential to make good decisions, something strong mentorship can help with greatly.

Q3 You have spoken before of the importance of networking, and how ESMO plays a key part in this. Were these same networking opportunities present earlier on in your career?

This is one of the main objectives of the Young Oncologists Committee: to make possible fruitful and lasting interactions. This is especially

important as, when you are young, something that can be lacking from within the field is networking opportunities, making this something that we can all work on. The Committee have created different programmes to bolster networking among young oncologists, and we have also been at work improving this at the congresses.

One of our initiatives is the young oncologists track, where you can attend sessions and interact with your peers that are also interested in your area of expertise. We have also the ESMO fellowships, in which you have the opportunity to visit another institution and work there; these always create networks because you can strive to maintain the relationship throughout the years. Facilitating the networking of our youngsters through initiatives such as these is possibly one of the most prominent roles of the Young Oncologists Committee.

Q4

Of the numerous exciting sessions presented at this year's ESMO Congress, which one were you most captivated by, or perhaps has implications for the work that you yourself are carrying out?

Some of the most important sessions were of course the presidential sessions, especially some of the different data presented on ovarian cancer. Additionally, new immunotherapy options appear to be set to change the current treatment of many cancers. The beacon data for *BRAF* mutant colorectal cancer also deserves special mention. These patients often have a very bad prognosis and usually do not respond to standard therapies. With these targeted therapies, however, you can really see changes in these patients. I must stress though that with so many interesting presentations at the Congress, it is difficult to pick just one.

Going back to the Young Oncologists Committee, one thing we are doing is to try and implement a session aimed at helping navigation of the Congress. This involves meeting with young people at the start of every single day and telling them the highlights of the coming day to better help them navigate the Congress.

Q5

You chaired a session at ESMO aimed at promoting gender balance across the oncology landscape. What made you interested in getting involved with this discussion?

That is correct. This arose due to the fact that Solange Peters, who is the chair of the Women for Oncology Committee, is also the society's president-elect, and in this role she is additionally a mentor to the Young Oncologists Committee. An opportunity for collaboration presented itself due to my role as chairman and hers as a mentor. We came to realise that one situation in the current panorama of medical oncology that required increased emphasis and attention was related to the young women in the field.

This is a common focus between the Women for Oncology Committee and the Young Oncology Committee, and has led to a collaboration in several projects with the aim to try and improve things for this population. We began including some of the sessions from the Women for Oncology Committee in our track, and vice versa, and based on this we decided that It made sense for me to chair this special session with her. The dissemination of information and changing of practice ultimately starts from the young people, making this a potentially very exciting partnership.

Q6

Your research interests have shifted to include immunotherapy and the identification of new biomarkers for colorectal cancer. How important are these lines of investigation in the oncological field?

Colon cancer still requires a lot of work. While other tumours with bad outcomes in the past have progressed far in terms of patient outcomes, colon cancer is somewhat stifled. We have very good current chemotherapy options, however are still faced with problems pertaining to molecular therapeutics and immunotherapy. Right now, we are dealing with very small populations of patients benefitting from this treatment, but for the vast majority of patients we do not have a bona fide option besides chemotherapy. Considering that colon cancer is a big killer, and is increasingly common across the world, it remains a significant problem to be faced.

Increased research into the identification of new biomarkers and new therapeutic targets, as well the design of new cancer immunotherapy strategies, is crucial towards improving the outcomes for these patients. I think it is one of the most the main challenges for medical oncology right now, and that more effort is needed.

Q7

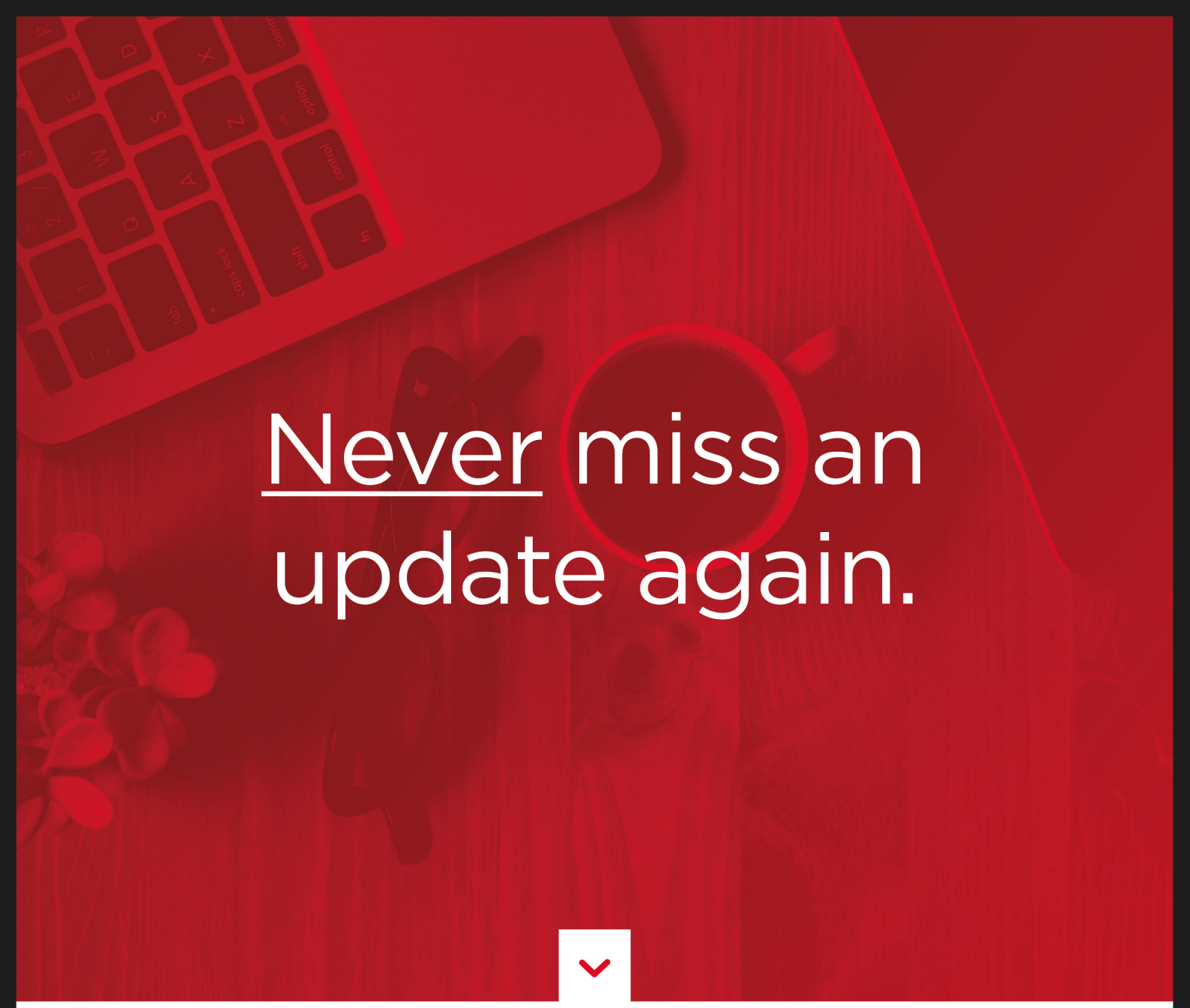
What are you most looking forward to at next year's Congress? Can we expect more field-defining advancements?

My personal opinion is that no one knows! But of course, immunotherapy will continue progress. Perhaps the discovery of new biomarkers to stratify patients to new combinations of agents besides the classics, e.g., PDL-1 expression, tumour mutational burden, and lymphocyte infiltration. We need to find more biomarkers and I think that in this regard, perhaps gene signatures, flow cytometry, and multiplex immunostaining approaches could make a difference.

Q8

Finally, are there any lessons that the more experienced members of the oncology committee could learn from their younger peers?

I think adaptability and flexibility, because the medical oncology field is changing all the time, from year to year. You can be more flexible when you are young, with the contrary being that when you are senior you have been working in the same field for years; once you reach a certain position, It's very difficult to adapt to a fast and constantly changing field like medical oncology. In summary, I think that the best lesson to be learnt is to be open-minded, flexible, and to be able to adapt to changes.



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Inflection Point: Novel Treatment Options are Forcing Next-Generation Sequencing into Standard of Care for Molecular Profiling in Oncology

An Update from the
European Society for Molecular Oncology (ESMO) Congress 2019

Chairpeople: European Society for Molecular Oncology (ESMO)

Speakers: European Society for Molecular Oncology (ESMO)

Acknowledgements: Medical writing by Christina Ranft Bernasconi and Luca Quagliata.

Support: The publication of this article was funded by Thermo Fisher.

Citation: EMJ Oncol. 2019;7[1]:30-36.

Meeting Summary

While precision medicine in oncology is eventually turning into reality outside the confined space of lung tumours, the approval of pan-cancer drugs, such as neurotrophic receptor tyrosine kinase (NTRK) inhibitors, is fostering the need for robust and reproducible molecular testing, in order to accurately identify treatment-eligible patients. At this year's European Society for Molecular Oncology (ESMO) congress in Barcelona, Spain, gathering >30,000 healthcare professionals spanning a range of disciplines and stakeholder groups, and >500 invited speakers, the latest update of clinical trial data showed the power of combining treatments, concurrently addressing multiple molecular pathways, and using both immune-oncology agents and targeted therapy. Similar to the 2018 congress, the integration of molecular data in the clinical management of cancer patients has been a major source of debate among specialists.

A number of workshops, satellite events, and new product launches at the ESMO congress were accompanied by dedicated companion diagnostic discussions. Most of the novel treatment options, either being new agents or therapeutic schemes, with sequential drug exposure and dosage adjustments, were complemented by presentations focussed on the need for adequate molecular testing. A few critical factors have emerged as being necessary for appropriate development and uptake of molecular profiling on a large scale in order to significantly impact patient outcomes.

Biomarkers Actionability

With a sustained number of new drugs or combination drugs entering standard of care this year, molecular testing should be constantly tailor based on those and should include the most updated relevant biomarkers to aid the best clinical decisions. In many panel discussions, a clear consensus was built around the need of triaging patient molecular and clinical data and to discuss in-depth the findings at local molecular tumour boards as a key element to enroot a truly personalised care model.

Conversely, a large and unresolved debate took place concerning to what extent molecular profiling should be used. Some major key opinion leaders advocated for the introduction of very

large next-generation sequencing (NGS) panel-based molecular testing including hundreds of genes, with many of those still having limited to no clinical actionability but holding the promise of increasing the patients' enrolling chances into clinical trials. On the other hand, a more consistent part of the audience was in favour of dedicated NGS panel-based tests covering clinically relevant genes (in the range of around 50) as being a more pragmatic and cost-effective approach.[1] The debate was further polarised between the standpoint of large academic centres versus community regional hospitals, having different resources both in terms of infrastructure and dedicated personnel. Given the complexity of cases to be analysed, it is evident that no one-size-fits-all solution exists because cancer type, molecular heterogeneity, the underlying clinical setting, and overall healthcare providers vary in terms of oncology patient support and management.[2]

Tumour Tissue Requirements along with Turnaround Times, from Sample Collection to Results, are More Critical than Ever Before

It is imperative that exhaustive biomarker testing results are available within days and not weeks before returning to the clinician. In fact, with many institutions now facing an increased pressure to deliver results leading to targeted therapy-related decisions, a clear trend in building in-house sequencing facilities to reduce time to result was at the forefront at this year's ESMO. Immuno-oncology agents are playing a pivotal role in underlining the need for fast testing procedure. In fact, a recurrent in practice scenario contemplates the initiation of an immuno-oncology drug regimen based on fast immunohistochemistry test results (i.e., programmed death-ligand 1 [PD-L1] positivity >1%) even before the mutational status of genes such as *EGFR* are known. Unfortunately, this fact leads to a number of mistreatments, especially for patients that after completing genomic testing turn out to be eligible for targeted therapy (e.g., *BRAF* positive melanoma).

In addition, in many of the discussions around molecular testing, the need for minimal tissue sample (i.e., working with cytological specimens) starting material and limited rejection rate (e.g., due to QNS) have turned out to be a basic requirement for any test to be broadly introduced into routine clinical practice. Any possible precaution should be taken in order to avoid a rebiopsy; anything that comes with associated risks, elevated costs and treatment delays, or when not applicable can lead to suboptimal therapy selection.

Molecular Testing Harmonisation

With many players and vendors now entering the molecular diagnostic field, the range of assays available on the market is steadily increasing; however, not all tests are born equal. In fact, there is a great need for harmonisation in nucleic acid testing in oncology. For instance, the range gene target number for molecular profiling across different assays and the absence of standard reference materials contributes to variability in test results among laboratories. A pivotal example is the recent unresolved issue related to the tumour mutational burden (TMB) assessment. Clinical studies have established TMB as a possible predictive biomarker for clinical efficacy of immune checkpoint inhibitors. However, there is a clear lack of standardisation for TMB estimation and reporting, something that is critical for ensuring reliability for its routine clinical implementation. An international effort to address this problem is currently lead by Friends of Cancer Research and Qualitätssicherungs-Initiative Pathologie GmbH (QuIP). Friends and QuIP aim to establish recommendations for achieving consistency in TMB estimation and reporting. Preliminary data from both stakeholders indicate several components to influence TMB estimation: preanalytical factors (e.g., input material quality/quantity), sequencing parameters (e.g., enrichment technologies), library preparation, bioinformatics (e.g., filtering of germline variants), as well as FFPE-induced deamination artefacts.

Such initiatives are necessary to assure that molecular testing can effectively enable true precision oncology by generating robust, reproducible, and meaningful data to inform treatment decisions.

Biomarkers for Immuno-Oncology Treatment Selection in Lung Tumour: An Open Debate

Nowadays, personalised oncology cannot be discussed without non-small cell lung cancer (NSCLC) as a pivotal example. With a fast-growing number of predictive biomarkers to be screened, but within the constraints of doing it in a tissue conservative manner, lung cancer represents both a great opportunity for new discovery and a challenging scenario for genomic profiling. Sequential testing algorithms are superseded by newer techniques such as NGS, being able to simultaneously look at a variety of biomarkers while only requiring low tissue input. In the field of immunotherapy, PD-L1 testing again played a major role at this year's ESMO congress as the most important biomarker to predict response to immune checkpoint inhibitors.[3] On the other hand, the controversial role of tissue TMB (tTMB) was not cleared up during the congress, with many conflicting data. The most debated study concerned the results from KEYNOTE-010 (tTMB available data for 253 patients) and KEYNOTE-042 (tTMB available data for 793 patients), including pembrolizumab versus chemotherapy in advanced NSCLC with mixed histology and a PD-L1 tumour proportion score (TPS) ≥1%.[4] tTMB status was defined with a cut-off point of 175 mutations/exome derived from a metanalysis of clinical trials across multiple tumour types. The chemotherapy arm showed no association with tTMB status; however, in the pembrolizumab arm a high tTMB value (i.e., TMB≥175) was associated with overall survival (OS), progression-free survival (PFS), and objective response rate. Conversely, Paz-Ares et al.[5] showed no association between tTMB and patient outcomes in pembrolizumab plus platinum-based chemotherapy for advanced untreated NSCLC with mixed histology. The patient cohort was composed of half of the patients of each of the KEYNOTE-021, KEYNOTE-189, and KEYNOTE-407 trials.

Overall, presented results indicated that tTMB requires careful re-evaluation as a biomarker for combination therapies, whereas the relationship for monotherapy has been confirmed in previous studies.[6] Among the unresolved crucial points, the definition of a universal TMB cut-off value (e.g., TMB ≥175 mutations/exome) seemed unrealistic, given that accumulating evidence suggests TMB to be highly tumour-type dependent. It was overall highlighted that further predictors for checkpoint inhibitor response need to be investigated, including immune infiltration scores and T-cell receptor clonality.

Overall, these results pinpoint the importance of determining the tumour mutational status at diagnosis as part of a board molecular profiling, in order to select the most appropriate treatment option for lung cancer patients.

Targeted Therapy Further Solidifies in Lung Tumour: Tyrosine Kinases Evolving Scenario

Outside the immunotherapy space, osimertinib was confirmed as an extremely effective option for first-line treatment of *EGFR*-mutated NSCLC based on the final data from the FLAURA trial,[7] moving the field forward from the original osimertinib scope as a third-line treatment for *T790M*-mutated patients. Improvement in PFS compared to first-generation EGFR-receptor tyrosine kinase inhibition was established, with a notable benefit especially in patients with brain metastases. Optimal sequencing regimens using tyrosine kinase inhibitors need to be further explored as resistance after osimertinib is observed and not yet fully understood. In the same study, ctDNA was analysed to monitor patients' disease progression. Mutational changes were visible before clinical progression was evident by monitoring for *T790M* or *C797S* resistance *EGFR* mutations during and after treatment.[7]

Outside EGFR, anaplastic lymphoma kinase positive NSCLC with active brain metastases now have an additional option given that the ASCEND-7 trial has confirmed ceritinib as a standard treatment option for those patients.[8] Of note, ASCEND-7 supports the activity of anaplastic lymphoma kinase inhibitors for brain metastases when administered prior to brain radiotherapy, thus allowing radiotherapy, along with its potential side effects, to be delayed.

Poly (ADP-Ribose) Polymerase Inhibitors move into First-Line in Ovarian and Breast Cancers: The Prominent Role of Breast Cancer Gene and Homologous Recombination Deficiency

Practice-changing Phase III trials were presented at the congress for newly diagnosed advanced ovarian cancers, wherein poly (ADP-ribose) polymerase (PARP) inhibitors are playing a major role. After the SOLO1 data presentation at ESMO 2018 in Munich, Germany, olaparib has demonstrated improved PFS in women newly diagnosed with high-grade advanced ovarian cancer with *BRCA1/2* mutation or homologous recombination deficiency (HRD).[9] The PRIMA/ENGOT-OV26/GOG-3012 trial highlighted that maintenance niraparib followed by platinum-based chemotherapy significantly extended PFS compared with placebo in the overall trial population (median: 13.8 months versus 8.2 months).[10] PARP inhibitors also elicit clear benefits to all newly diagnosed advanced ovarian cancers, independently from *BRCA1/2* status alone or in combination with chemotherapy or bevacizumab.[11] Olaparib plus bevacizumab significantly improved PFS compared with placebo plus bevacizumab in the overall population (median: 22.1 months versus 16.6 months), regardless of *BRCA* mutation status. However, in patients with *BRCA*-mutated tumours, olaparib plus bevacizumab was associated with superior PFS (median: 37.2 months versus 21.7 months), but with less benefit in patients with non-*BRCA*-mutated tumours (median: 18.9 months versus 16.0 months). Notably, there appeared to be no significant benefit for olaparib plus bevacizumab as maintenance regimen in patients with negative or unknown HRD status (median: PFS 16.9 months versus 16.0 months). However, the clinical validity of testing for HRD status needs to be comprehensively investigated.

In the BROCADE3 trial, Huggins-Puhalla et al.[12] showed that patients with advanced human *EGFR2*-negative breast cancer and germline *BRCA* mutation demonstrate significantly improved PFS with the addition of the PARP inhibitor veliparib to chemotherapy over placebo plus chemotherapy.

Yet another example highlighting the importance of a correct *BRCA* assessment, underlining the importance and the need to ramp up molecular diagnostics capabilities in the current scenario of patient management.[13]

Novelty in Colorectal Cancer: Beyond *BRAF V600E*

Most updated results from the BEACON trial[14] show unmatched OS in the second-line treatment of metastatic colorectal cancer (CRC) positive for *BRAF V600E* with a combo of three targeted agents, namely encorafenib, cetuximab, and binimetinib. The results of the randomised Phase III study based on 444 patients showed that the triplet combination was associated with markedly superior median OS (9.0 months versus 8.4 months) and objective response rate (26% versus 20%), compared with the doublet (encorafenib with cetuximab). Additionally, patients with *BRAF V600E*-mutated CRC benefited from surgery of liver metastases. In a retrospective series of 91 patients with *BRAF V600E*-mutated CRC and liver-only metastases, multivariate analysis found that surgery was associated with significantly longer OS and PFS than a chemotherapy-only strategy.[15]

Overall, the presented data emphasise the value of assessing *BRAF* mutations, not just *V600E*, outside the most commonly tested space of melanoma, and demonstrate the important predictive value of *BRAF* in patients with advanced CRC.

Biliary Tract Cancer: Time for Molecularly Informed Treatment Decisions

Biliary tract cancers, especially intrahepatic cholangiocarcinoma, often present (≤40% of cases) with fibroblast growth factor receptor (*FGFR*) 2 gene fusions along with isocitrate dehydrogenase-1 (*IDH1*) mutations. Pemigatinib, a FGFR inhibitor with compelling clinical efficacy in patients having *FGFR2* gene rearrangements or fusions, was presented at ESMO. Pemigatinib achieved an objective response rate of 35.5% and a median response duration of 7.5 months,

along with median PFS and OS of 6.9 months and 21.1 months, respectively.[16] Derazantinib and infigratinib, other FGFR inhibitors, further showed encouraging results in early-stage clinical trials (Phase IIa studies).

Patients with advanced cholangiocarcinoma presenting *IDH1* mutations and treated with IDH1 inhibitor ivosidenib in a Phase III clinical trial (ClarIDHy) showed some clinical benefit compared to placebo.[17] The Phase III trial confirms that targeting *IDH1* mutations in cholangiocarcinoma is a promising strategy, but the debate is open on whether results are clinically meaningful. Overall, *IDH1* mutations remain a highly interesting target for cholangiocarcinoma treatment.

The above-mentioned studies demonstrate that precision medicine in advanced cholangiocarcinoma has finally started to gain traction. Tumour profiling should be taken into consideration to decide upon treatment options and should become a new standard for patients diagnosed with advanced cholangiocarcinoma.

Prostate Cancer: *BRCA* Gene and Homologous Recombination Deficiency Status are Changing the Treatment Scenario

The PROfound study[18] results showed a clinically meaningful benefit in radiological PFS with olaparib in metastatic castration-resistant prostate cancer (mCRPC) with *BRCA1, BRCA2*, or ataxia-telangiesctasia mutated genes.[18] PROfound evaluated the efficacy and safety of olaparib versus enzalutamide or abiraterone in 387 patients with mCRPC who have failed prior treatment with a new hormonal agent and have a tumour mutation in one or more of 15 genes involved in the homologous recombination repair (HRR) pathway. Remarkably, the PROfound trial is the first positive Phase III biomarker-based (i.e., HRR) study in mCRPC.[19] Olaparib reduced the risk of progression by 66% ($p<0.0001$) in patients with alterations in *BRCA1, BRCA2*, or ataxia-telangiesctasia mutated gene by 51% ($p<0.0001$) in patients with alterations in any qualifying HRR gene.

As for ovarian and breast cancers, it is pivotal to rapidly equip molecular pathologists with the appropriate solutions to effectively test for HRR-related genes in order to inform treatment decisions.

Liquid Biopsy for Routine Testing: Towards Real-Time Disease Monitoring

Within the context of the FLAURA trial,[7] results presented at ESMO from an exploratory analysis using circulating tumour DNA (ctDNA) to monitor patients with *EGFR*-mutated NSCLC showed that early detection of disease progression is feasible, and that mutational changes can be detected in ctDNA before clinical progression is evident.[7] In detail, the detection of *ex19del, L858R*, or *T790M EGFR* mutations via plasma-derived ctDNA analysis was performed before, during, and after treatment. *T790M* or *C797S* resistance mutations were monitored during and after treatment. Notably, of the 122 patients who had their ctDNA monitored, progression according to ctDNA data preceded or occurred concurrently with manifest clinical disease progression in about 66% of patients, with a median lead time of 2.7 months. Acquired *EGFR C797S* or *T790M* resistance mutations were detected in 8% and 74% of patients with ctDNA progression in the osimertinib and comparator arms, respectively. Earlier awareness that resistance is present and prompt identification of the driving mutation might impact the overall therapy management process.[20]

Considering the fast-evolving technical progress enabling testing at increased sensitivity and specificity, liquid biopsy is emerging as a valuable diagnostic tool, including for minimal residual disease monitoring to determine treatment success in early-stage cancers. At the congress, several presented studies used liquid biopsies as an assessment tool in the prediction of prognosis for CRC. The IDEA FRANCE study investigated Stage III colon cancer and assessed the risk of 3-month adjuvant chemotherapy treatment versus 6-month standard treatment using ctDNA as a selecting factor, demonstrating the value of ctDNA analysis. However, the trade-off between monitoring a patient's individual mutations in a specifically designed single analyte liquid biopsy test

compared to using a broader NGS-gene panel comes down to difference in the assay sensitivity, a parameter that is vital for a proper minimal residual disease monitoring.[21] Overall, the utility of liquid biopsy for MDR detection has not been fully proved, but this year's data posed an important milestone toward this goal.

Molecular Diagnostics: Why Build an Interdisciplinary Approach

Molecular tumour boards (MTB) are an emerging entity in the field of oncology. A multidisciplinary approach enabling physicians to cope with individual patient history and to provide genotype matching opportunities is highly needed.[22] MTB is also a forum for continuing education and to increase oncologists' confidence in making treatment options while disseminating updates around molecular testing. The following specialists should be included in an MTB: oncologists, pathologists, geneticists and genetic counsellors, bioinformaticians, radiologists, and basic scientists to give insight on individual pathways and drug access specialists to provide information about ongoing clinical trials. The biggest challenges are that not all hospitals and practices have access to such a structure, and there is a lack of availability of fitting clinical studies in all geographic area.

Genomic testing increases the ability to find opportunities for patient treatment and generates a large amount of clinical data that can be used for translation research discoveries. To facilitate and aggregate such a mass of data, there is an increasing need to have software solutions that would facilitate NGS data interpretation to narrow down actionable mutations and help to focus MTB discussion. Furthermore, it is important that data collected by different stakeholders is accessible anywhere and that includes the outcomes of MTB discussions. This would also require an appropriate framework in regard to data sharing and acquisition, where major international associations, like ESMO and American Society of Clinical Oncology (ASCO), should play a prominent role in process governance.

Conclusion

While there has been a tremendous improvement around treatment availability as demonstrated during the ESMO congress, there is still a major hurdle hampering the prompt transfer of these therapies to patients: access to timely molecular testing results. For these drugs to benefit more patients, there needs to be a major paradigm shift in the way genomic patient tumour profiles are generated, interpreted, and provided to oncologists, particularly in the community hospital setting. It is now clear that in order to expedite access to the full arsenal of available targeted therapies, NGS will have to become mainstream.

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Abstract Reviews

In the following summaries, presenters of some of the most exciting abstracts and posters from the congress deliver their research to you in their own words.

A Novel Affinity-Enhanced NY-ESO-1-Targeting TCR-Redirected T cell Transfer Exhibiting Early-Onset Cytokine Release Syndrome and Subsequent Tumour Responses in Synovial Sarcoma Patients

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Disclosure: Dr Ishihara has received personal fees from Chugai, Eisai, MSD, Pfizer, and Takara Bio. Dr Yamamoto has received grants from Astellas, Bayer, BMS, Boehringer Ingelheim, Chugai, Daiichi-Sankyo, Eisai, Janssen Pharma, Kyowa-Hakko Kirin, Lilly, Merck, Novartis, Pfizer, Quintiles, and Taiho; and personal fees from AstraZeneca, BMS, Boehringer Ingelheim, Chugai, Cimic, Eisai, Lilly, Ono Pharmaceutical Co. Ltd., Otsuka, Takeda, Pfizer, and Sysmex. Dr Kojima has received grants from Astellas Amgen BioPharma, Chugaiseiyaku, MSD, Oncolys BioPharma, Ono Pharmaceutical Co. Ltd., Paraxel, and Sihonogi. Dr Sasada has received grants from AMED, BrightPath Biotherapeutics Co. Ltd., JSPS, and Taiho; and personal fees from Bristol-Myers Squibb, Chugai, Nippon Kayaku, and Ono Pharmaceutical Co. Ltd. Dr Okamoto, Dr Tomura, Dr Chono, Dr Nukaya, and Dr Mineno have received grants from AMED and personal fees from TakaraBio Inc. Dr Ikeda has received grants from Takara Bio Inc. Dr Watanabe has received personal fees from AstraZeneca, Bristol-Meyers Squibb, and Chugai; and has funding source to

support Dept. of Immuno-Gene Therapy in Mie Univ. from TakaraBio Inc. and United Immunity Inc. Dr Skiku has received grants from TakaraBio Inc. and licensed patents from Virus Vector.

Acknowledgements: The authors are grateful to members of TakaraBio Inc. for the preparation of TCR-T-cells and the analysis of the cell kinetics, and also to members of Takara Bio Inc. and FiveRings Co. for their support to the clinical trial management. This research was supported by the Medical Research and Development Programs Focused on Technology Transfer, Adaptable and Seamless Technology Transfer Program Through Target-driven R&D (A-STEP) from Japan Agency for Medical Research and development (AMED).

Keywords: Cytokine release syndrome (CRS), NY-ESO-1, synovial sarcoma, T cell receptor (TCR)-redirected T cell transfer, tocilizumab.

Citation: EMJ Oncol. 2019;7[1]:38-40. Abstract Review No: AR01.

BACKGROUND

Adoptive transfer of T cell receptor (TCR)-redirected T cells has been reported to exhibit efficacy in some patients with melanoma and sarcoma.[1] However, cytokine release syndrome (CRS) and its relation to tumour response has not been well-documented. This study aimed to evaluate clinical responses in association with the cell kinetics and CRS after transfer of high-affinity NY-ESO-1 TCR-gene transduced T cells in cancer patients.[2]

METHODS

The authors developed a novel-type affinity-enhanced NY-ESO-1-specific TCR and an originally-developed retrovirus vector that encodes small interfering RNA (siRNA) to silence endogenous TCR creation.[3] The NY-ESO-1/TCR sequence was mutated for high affinity with replacements of G50A and A51E in the CDR2 region.[4] This was a first-in-human clinical trial of the novel NY-ESO-1-specfic TCR-T cell transfer to evaluate the safety, *in vivo* cell kinetics, and clinical responses. It was designed as a cell-dose escalation from $5x10^8$ (cohort 1) to $5x10^9$ (cohort 2) cells. NY-ESO-1-expressing refractory cancer patients were enrolled with a 3+3 cohort design. Eligibility criteria included being ≥20 years of age, recurrent/refractory tumour, NY-ESO-1 positive expression in the tumour specimen, *HLA-A*02:01* or **02:06* (+) for NY-ESO-1, and informed consent. A 200 mL blood draw from each patient was obtained. TCR-gene transduction and culture were carried out for 10–12 days, followed by deep freezing quality check. Cyclophosphamide (1,500 mg/m^2) was administered prior to the TCR-T cell transfer as preconditioning.

RESULTS

Nine patients were treated with the TCR-T cells that expanded in peripheral blood with a dose-dependent manner, associated with rapid proliferation within 5 days of infusion. Three patients receiving $5x10^9$ cells developed early-onset CRS, with elevated levels of serum IL-6 and IFN-γ. These CRS on Day 1 or 2 were well managed with tocilizumab treatment. Three synovial sarcoma patients exhibited tumour shrinkage and partial responses, and they all had high-expression of NY-ESO-1 in the tumour samples, namely 75% or more (Table 1). Intriguingly, tumour regrowth seemed to be inversely correlated with a steep decrease in the number of the TCR-gene-transduced lymphocytes after the TCR-T therapy, especially in the patient with high tumour burden, such as TBI1301-16. Exploratory analysis revealed that multiple chemotactic cytokines, including CCL2 and CCL7, as well as IL-3 increased in the serum of patients with CRS. The proportions of effector-memory phenotype T cells in the infused cell-product were significantly associated with CRS development.

CONCLUSION

The affinity-enhanced NY-ESO-1/TCR-T cell transfer exhibited early-onset CRS in association with *in vivo* cell proliferation and sequential tumour responses in the patients with high-NY-ESO-1-expressing synovial sarcoma. Further understanding and development of the TCR-T therapies are needed to increase the ability to overcome the challenges of treating solid tumours, such as high tumour burden patients.

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Table 1: Patients' characteristics who received adoptive transfer of TBI-1301.

Cohort	Patient ID	Age	Sex	Cancer type	Tumour lesions at entry	CRS	NY-ESO-1 expression (%)	Best tumour response
1	TBI1301-01	67	F	Breast cancer	Lung, lymph node	(-)	5–20	PD
1	TBI1301-02	40	F	Synovial sarcoma	Lung	(-)	>75	SD**
1	TBI1301-03	73	M	Malignant salivary tumour	Primary lesion at parotid gland	(-)	<5	SD
2	TBI1301-07	46	M	Synovial sarcoma	Soft tissue at femoral area, lung	(-)	>75	PR
2	TBI1301-09	61	M	Melanoma	Skin, liver, peritoneum	CRS (G2)*	>75	SD
2	TBI1301-08	70	M	Synovial sarcoma	Chest wall, soft tissue at inguinal area, bone	CRS (G2)*	>75	PR
2	TBI1301-14	65	F	Ovarian cancer	Lymph node	(-)	25–50	SD**
2	TBI1301-16	25	M	Synovial sarcoma	Lung	CRS (G2)*	>75	PR
2	TBI1301-15	45	F	Myxoid cell liposarcoma	Retroperitoneum	(-)	50–75	SD

*Tocilizumab was used to treat CRS. **Cases without measurable lesions.
CRS: cytokine release syndrome; PD: progressive disease; PR: partial response; SD: stable disease.

A Phase II Study of Pazopanib as Front-Line Therapy in Patients with Nonresectable or Metastatic Soft Tissue Sarcomas Who are Not Candidates for Chemotherapy

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Disclosure: Dr Agulnik has received consulting fees from Novartis, Lilly, Immune Design, and Bayer; and Speaker's Bureau from Janssen, Eisai, BMS, and Bayer. Dr Monga has received travel funding from Deciphera; and has received research funding from Immunocellular and Orbus Therapeutics. Dr Milhem has been a Consultant or on the advisory boards for Amgen, Trieza, Biontech, Blueprint Medicine, Immunocore, and Array BioPharma, Inc. Dr Robinson has received research support from TRACON Pharmaceutical; has been on the advisory boards for BTG International and the Society of Interventional Radiology Foundation; and has received honoraria to institution (all outside the submitted work). Dr Attia has received research funding from Desmoid Tumor Research Foundation; has received institute research funding from AB Science, TRACON Pharma, CytRx Corporation, Bayer, Novartis, Daiichi Sankyo, Lilly, Immune Design, Karyopharm Therapeutics, Epizyme, Blueprint Medicines, Genmab, CBA Pharma, Merck, Philogen, Gradalis, Deciphera, Takeda, Incyte, Springworks, Adaptimmune, Advenchen Laboratories, Bavarian Nordic, BTG, PTC Therapeutics, GlaxoSmithKline, and FORMA Therapeutics; and has received travel, accommodations, and expenses from Immune Design. Dr VanTine has received unrelated basic science grant funding from Pfizer, Tracon, and Merck; has received consulting fees from Epizyme, Lilly, CytRX, Janssen, Immune Design, Daiichi Sankyo, Plexxicon, and Adaptimmune; has received speaking fees from Caris, Janssen, and Lilly; and has received travel fees from Adaptimmune and Lilly. All other authors have declared no conflicts of interest.

Acknowledgements: This trial was funded by Novartis and run through the Midwest Sarcoma Trials Partnership.

Keywords: Clinical benefit ratio, elderly patients, pazopanib, Phase II, sarcoma.

Citation: EMJ Oncol. 2019;7[1]:41-43. Abstract Review No: AR02.

BACKGROUND

Patients with metastatic soft tissue sarcoma (STS) have a poor prognosis with a median survival of 12–14 months.[1] First-line therapy consists of anthracycline-based cytotoxic chemotherapy. Doxorubicin remains the most active single agent with a response rate of 25%.[2,3] The treatment of patients with advanced STS who are unsuitable for front-line cytotoxic therapy because of age, comorbidities, or poor performance status poses a treatment dilemma. Pazopanib is a multi-targeted tyrosine kinase inhibitor that is U.S. Food and Drug Administration (FDA) approved for second-line and beyond treatment for metastatic STS. Approval was based on the PALETTE study, a Phase III study of 372 patients with metastatic STS who had progressed on standard chemotherapy.

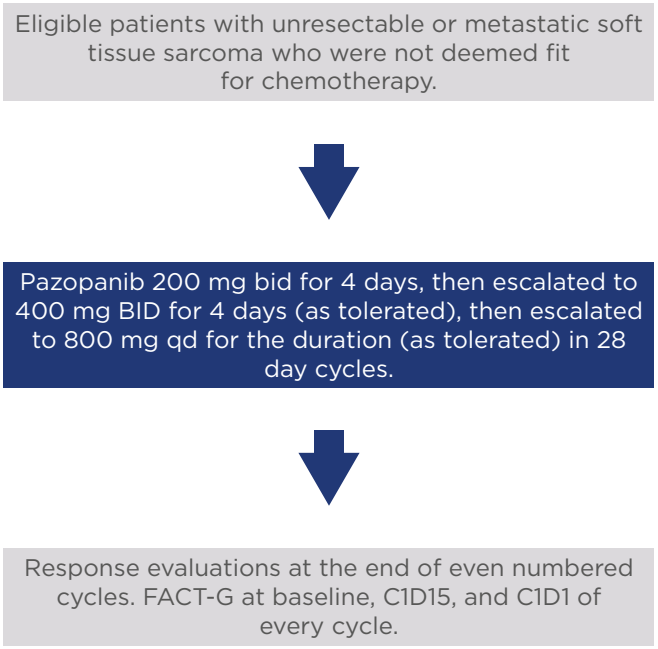

Figure 1: Study schema

In this trial, a median progression free survival (PFS) of 4.6 months in the pazopanib arm compared to 1.6 months in the placebo arm, and overall survival (OS) of 12.5 and 10.7 months, respectively, was observed.[4] Treatment was well tolerated; the most common adverse events were fatigue, diarrhoea, nausea, weight loss, and hypertension.[4] EPAZ, a noninferiority study comparing doxorubicin and pazopanib in the front line in patients >60 with nonresectable or metastatic STS, noted a PFS of 5.3 months for doxorubicin compared to 4.4 months for pazopanib (p=0.993), and OS of 14.3 and 12.3 months, respectively (p=0.735).[5] Herein, the authors report a Phase II study to evaluate pazopanib as a first-line agent in patients with nonresectable or metastatic disease who were not felt to be candidates for cytotoxic chemotherapy by the treating physician.

METHODS

Eligible patients had histologically confirmed nonresectable or metastatic STS, were at least 18 years old, not a candidate for chemotherapy as determined by the treating physician, and had not received prior systemic therapy for sarcoma. Initial starting dose of pazopanib was 200 mg twice daily and titrated to 800 mg daily (Figure 1). The primary endpoint was clinical benefit rate (CBR) (complete response + partial response + stable disease per RECIST 1.1) at 16 weeks. The sample size of 56 evaluable patients was calculated to provide 80% power to test a hypothesised CBR of ≥35% against an unfavourable CBR of ≤20%. If ≥17 patients achieved benefit, the null CBR of 20% would be rejected at a nominal 5% alpha level (actual alpha=0.043). Secondary endpoints included PFS rate, OS, quality of life, and serum biomarkers.

A total of 56 patients were included in the intention-to-treat analysis. The median age was 78.7 years (60–91 years), ECOG 0–2 (14% of patients ECOG 2), 82% of patients had metastatic disease. Histologic subtypes included liposarcoma (n=2), leiomyosarcoma (n=21), UPS (n=19), and other (n=14). The CBR was 37.5% (21/56), 95% Wilson confidence interval (CI): 0.2492–0.5145, 2-sided exact binomial test p=0.0019. An additional 17.5% of patients (eight stable disease, two partial response) could not be confirmed by a second scan, and 18% were not evaluable for best response (n=10). The median PFS was 3.67 (2.05–24.14) months, and PFS rate at 4 months was 44% (95% CI: 0.33-0.6). Median OS was 13.22 (95% CI: 8.46–not

reached) months. No new or unexpected adverse events were seen; the most common Grade I–II adverse events were diarrhoea, nausea, and fatigue. The most common Grade III-IV adverse events were hypertension and liver function test abnormalities. No change was seen in quality of life scores by drug treatment.

CONCLUSION

In patients for whom cytotoxic chemotherapy is not an option, treatment for STS is limited. The primary endpoint of this study was met with a CBR of 37.5%. These data suggest there is a benefit to front-line pazopanib in this patient population.

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The Mutational Signature of Spontaneously Developing Tumours in MLH1$^{-/-}$ Mice - Potential Consequences for Immunotherapeutic Approaches

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Disclosure: The authors have declared no conflicts of interest.

Acknowledgements: The authors gratefully thank Mrs Ilona Klamfuss for breeding MLH1 mice.

Support: This work was supported by the German research foundation to CM (DFG grant number MA5799/2-1 and MA5799/2-2).

Keywords: Gastrointestinal tract (GIT), exclusive/shared mutations, mismatch repair-deficient (MMR-D), non-Hodgkin T cell lymphoma (NHL), predicted tumour antigen, tumour mutational burden (TMB).

Citation: EMJ Oncol. 2019;7[1]:43-45. Abstract Review No. AR03.

INTRODUCTION

Mismatch repair-deficient (MMR-D) tumours exemplify the prototypic hypermutator phenotype. Owing to the high mutation rates, many neo-antigens are present on the tumour cells' surface, typically shared among different cancer types. The *MLH1* knockout mouse represents a preclinical model that resembles features of the human MMR-D counterpart.[1,2] These mice develop MMR-D neoplasias spontaneously.

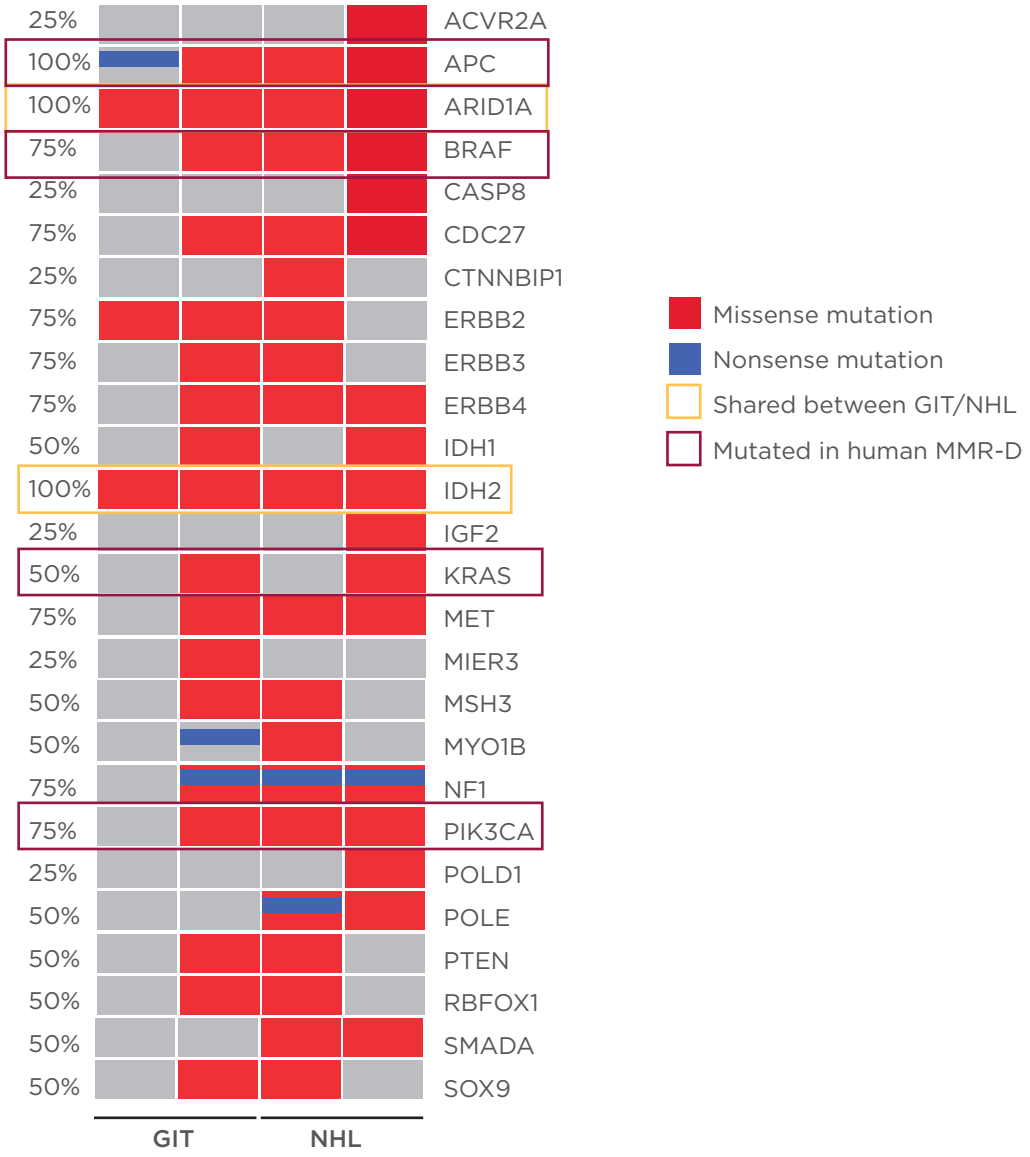

Figure 1: Mutational signature of MLH1$^{-/-}$-associated tumours in mice.

Oncoprint presenting genes with tumour suppressive function as well as relevance for tumour initiation and progression. Non-synonymous alterations are shown for particular genes (rows) affecting particular individual samples (columns). Shared mutations (orange box) as well as mutations conserved in the human counterpart (red box) are highlighted. Red bars indicate missense and blue bars nonsense mutations.

GIT: gastrointestinal tumour; NHL: non-Hodgkin lymphoma.

The tumour spectrum is complex, with a high prevalence of early non-Hodgkin T cell lymphomas (NHL), lymphoid skin lesions, and at later stages, epithelial tumours of the gastrointestinal tract (GIT). The sequential appearance of these neoplasias, often in a mutually exclusive fashion (the 'either-or' principle of tumour type), raises the question of how the biological and/or molecular mechanisms of the neoplasia relate to each other.

METHODS

Using whole-exome sequencing on MLH1$^{-/-}$ primary tumours (two GIT, one splenic NHL, and one skin lymphoma) as well as GIT-derived cell lines (n=2), the study aimed to identify underlying molecular alterations. The researchers focussed on shared and mutually exclusive mutations, and described the processes of ongoing mutational

events in tumour-derived cultures. Finally, the coding microsatellite mutational profile was examined on a panel of primary tumours to detect shared mutations in MMR-D target genes.

RESULTS

MLH1$^{-/-}$ tumours show high tumour mutational burden with three of four primary tumour samples being ultra-hypermutated (>100 mutations/megabase). Missense mutations were more frequent than nonsense mutations, and base changes were mainly due to transitions (C>T; A>G). The resulting mutational landscape was heterogeneous and in accordance with the human counterpart; MLH1$^{-/-}$ tumours frequently harbour mutations in *PIK3CA, EGFR, BRAF, KRAS*, and *ERBB3*.[1] Of note, only a few shared mutations were detectable among different tumour entities (*ARID1A* and *IDH2*). Mutations in the tumour suppressor genes *SMAD4* and *POLE* were mutually exclusive in lymphomas, most likely contributing to a more aggressive *in vivo* phenotype (Figure 1). *POLE* mutations affect the exonuclease domain, similar to the human MMR-D counterpart.[3,4] As a consequence, affected cells rapidly accumulate point mutations, yielding the ultrahypermutated phenotype. This was similar to the observations made in this study. Comparing the mutational profile of selected primary tumours, and their corresponding cell line, *in vitro* culture revealed continuously increased numbers of somatic gene mutations. The same was true for coding microsatellite mutations in selected MMR-D target genes, showing a gradual increase during *in vitro* passage. With respect to the latter mutation type, a partial overlap was detectable, and shared antigens were recognised. The two most promising candidates are *AKT3*, a RAC-gamma serine/threonine-protein kinase involved in the maintenance of cellular homeostasis, and *ERCC5* (Excision Repair 5, Endonuclease), involved in DNA excision repair. Mutations in this gene increase susceptibility for skin cancer development. Given the biological function of these two genes, their applicability as a target is worth determining.

CONCLUSION

This pilot study is the first reporting results of a comparison between different spontaneously developing tumours as models for MMR-D driven tumourigenesis. *ARID1A* was identified as a potential secondary causative mutation hotspot, which is in line with the human counterpart associated with shortened time to cancer-specific mortality. This comprehensive characterisation of the mutational landscape may, therefore, be a good starting point to predict antigens for vaccination approaches.

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The 2016-2019 ImmunoTOX Assessment Board Report: Results from a Descriptive Real-Life Study of a Collaborative Management of Immune-Related Adverse Events Induced by Immune Checkpoint Inhibitors

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Disclosure: Principal/sub-Investigator of clinical trials for: Abbvie, Aduro, Agios, Amgen, Argen-x, Astex, AstraZeneca, Aveo pharmaceuticals, Bayer, Beigene, Blueprint, BMS, Boeringer Ingelheim, Celgene, Chugai, Clovis, Daiichi Sankyo, Debiopharm, Eisai, Eos, Exelixis, Forma, Gamamabs, Genentech, Gortec, GSK, H3 biomedecine, Incyte, Innate Pharma, Janssen, Kura Oncology, Kyowa, Lilly, Loxo, Lysarc, Lytix Biopharma, Medimmune, Menarini, Merus, MSD, Nanobiotix, Nektar Therapeutics, Novartis, Octimet, Oncoethix, Oncopeptides AB, Orion, Pfizer, Pharmamar, Pierre Fabre, Roche, Sanofi, Servier, Sierra Oncology, Taiho, Takeda, Tesaro, and Xencor. Personal fees (money paid for services rendered, generally honoraria, royalties or fees for consulting, lectures, speakers bureaus, expert testimony, employment, ad-boards, etc.): Celgene, Bristol-Myers Squibb, AstraZeneca, and Janssen. Non-financial support (drugs, equipment supplied by the entity, travel paid by the entity, writing assistance, administrative support, etc.): AstraZeneca, Roche, Novartis, Gilead, Celgene, and Bristol-Myers Squibb.

Acknowledgements: The author would like to thank the patients, the patients' families, and all the study investigators and personnel. The author also thanks David Fraser (Biotech Communication SARL, Ploudalmézeau, France) for his precious and much appreciated copy-editing assistance. Lastly, the author thanks Janine Nda, Cécile Geniez, Stéphanie Demirdjian, and Sandrine Thorel for their assistance with patient management, and Alexandre Maria and Julie Perrin for their contributions and referral advice.

Support: Immune-related adverse event (irAE), multidisciplinary collaborative approach, patient care network.

Keywords: Immune-related adverse event (irAE), multidisciplinary collaborative approach, patient care network.

Citation: EMJ Oncol. 2019;7[1]:46-47. Abstract Review No. AR04.

BACKGROUND

Although immunotherapy is better tolerated globally than chemotherapy, immune checkpoint inhibitors may be associated with immune-related adverse events (irAE), characterised by their diversity, unpredictability, and corticosteroid responsiveness.[1,2] The author investigated the ImmunoTOX meeting's activities: a multidisciplinary real life and case-by-case approach to manage irAE and capture the salient medical needs in the management of immunological toxicities.

METHODS

The ImmunoTOX assessment board is an academic, multidisciplinary group of oncologists and organ specialists that was set up in April 2016 at Gustave Roussy cancer centre in France. The board meets every 2 weeks to discuss the case-by-case management of patients presenting with irAE. This report describes ImmunoTOX's meeting activities between 6th April 2016 and 2nd January 2019.

RESULTS

Over the 32 months of study period, 398 requests concerning 356 patients were submitted to the ImmunoTOX board. The most common tumour types of patients were thoracic cancers (n=105, 29%), skin cancers (n=82, 23%), and renal carcinomas (n=28, 8%). The requests most frequently asked were causal link between immunotherapy and adverse event (n=148, 37%), the possibility for retreatment after hold due to previous adverse event (n=109, 27%), the clinical management of complex situation (n=100, 25%), and the initiation of immunotherapy in patients

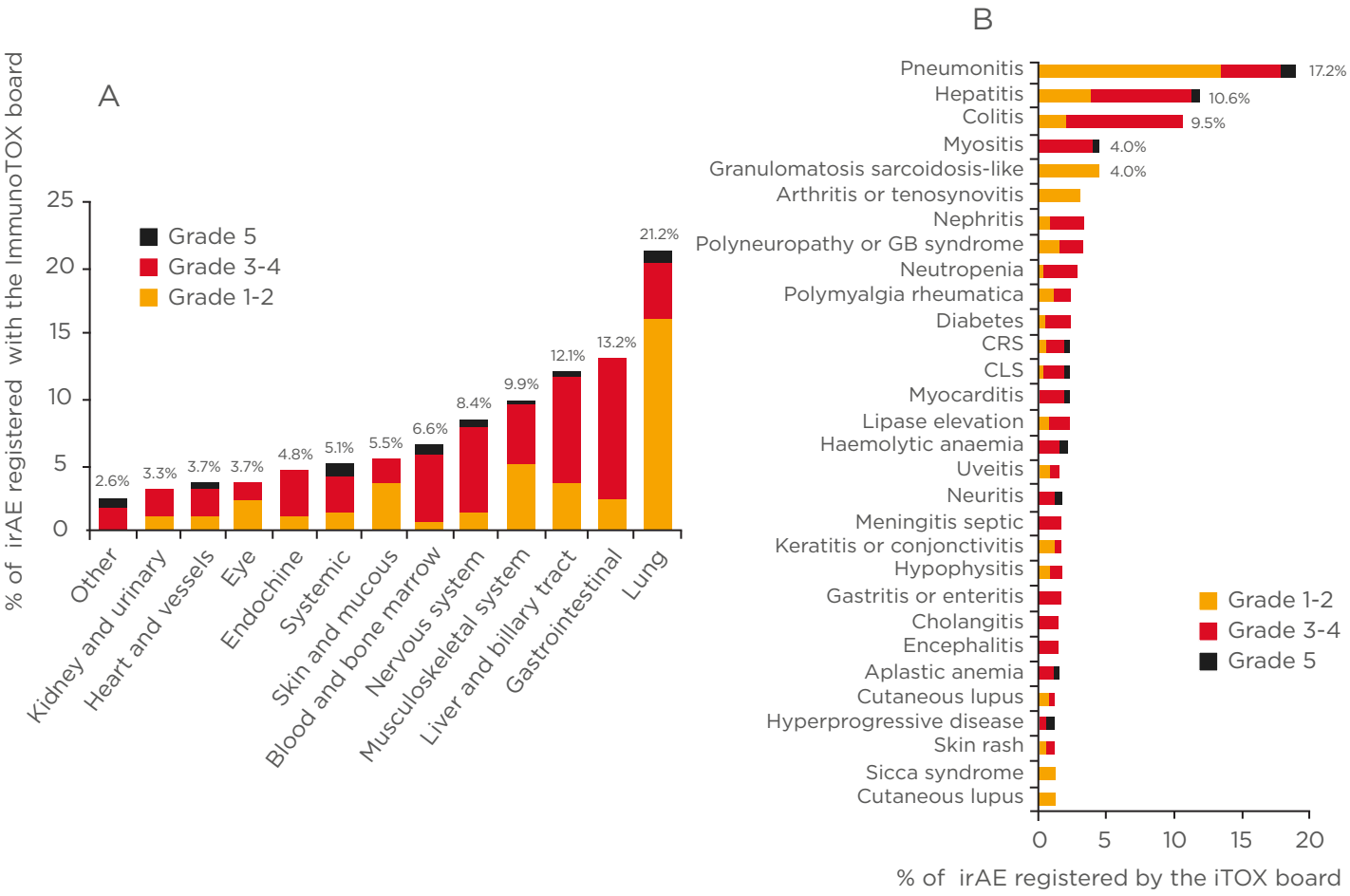

Figure 1: Distribution of irAE organ categories considered by the ImmunoTOX assessment board. In the figure, only irAE that occurred in three or more patients are shown.

CLS: capillary leak system; CRS: cytokine release syndrome; GB syndrome: Guillain-Barré syndrome; irAE: immune-related adverse event.

with pre-existing comorbidities (n=41, 10%). The ImmunoTOX board found a relationship between immunotherapy and adverse event in 273 (77%) of the 356 patients. The organ systems most frequently involved by irAE were the lung (n=58, 21%), gastrointestinal tract (n=36, 13%), liver or biliary tract (n=33, 12%), musculoskeletal system (n=27, 10%), and nervous system (n=23, 8%). The retreatment by immunotherapy after holding due to previous adverse event and the initiation of immunotherapy in patients with pre-existing autoimmune comorbidities were assessed as precaution for use and not formally contraindication in 65% and 93% of the cases, respectively.

CONCLUSION

The medical needs in the management of immune-related adverse events involves five salient organ systems, namely the lung, gastrointestinal, liver and biliary tract, musculoskeletal, and nervous systems. Retreatment after holding due to previous adverse events and immunotherapy initiation in patients with autoimmune comorbidities were mostly assessed as precaution for use and not formal contraindication. A multidisciplinary and case by case approach can be helpful and complementary of general guidelines in the management of immunological toxicities.

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Measuring the Cancer Burden in Europe: the European Cancer Information System (ECIS)

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Disclosure: The authors have declared no conflicts of interest.

Keywords: Cancer burden indicators, cancer incidence, cancer registries, European Cancer Information System (ECIS), European Network of Cancer Registries (ENCR), mortality, survival.

Citation: EMJ Oncol. 2019;7[1]:48-49. Abstract Review No: AR05.

Cancer is the second leading cause of mortality in European Union (EU) Member States,[1] with more than 1.4 million deaths estimated for 2018 across the 28 EU countries.[2] For more than 30 years since the first 'Europe against cancer' programme was launched, actions taken at the EU level[3] have helped to extend and save lives. Evaluation of the implemented measures and their effectiveness is critically dependent on accurate and comparable data allowing derivation of cancer indicators (i.e., incidence, mortality, survival): reliable high-quality information helps co-ordinate and improve cancer prevention across the EU via the promotion of good practices. In support of this process, the Joint Research Centre (JRC), acting in its scientific role to the European Commission and in close collaboration with the Commission's Directorate-General for Health and Food Safety (DG SANTE) as well as with major European stakeholders in the field, has been developing and is maintaining the European Cancer Information System (ECIS) as a comprehensive health and research infrastructure harmonising cancer registries' data and producing meaningful information to facilitate the interpretation of the dynamics of cancer in Europe.

Data needed to quantify the cancer burden in a geographically-defined population are systematically collected by population-based cancer registries (CR), which are the information source for all reportable cancer cases in the specific area. Since 2012, in response to the call from the European Council to the Commission to act further in harmonising EU cancer registration, the JRC has taken an active role in supporting the activities and exploiting the data of the CR affiliated to the European Network of Cancer Registries (ENCR), currently including 178 individual registries across Europe (comprising non-EU countries).[4] The contribution of the JRC consists not only in the harmonisation of CR data and registration processes, but also in the collection, validation, analysis, and dissemination of the cancer burden indicators computed from CR input data. The ECIS infrastructure has been developed by the JRC in recent years serving this purpose, consisting of several components to manage a central data repository and to co-ordinate in an efficient and sustainable way the activities of data quality control, analysis, and dissemination.

A key component of the system is the ECIS web application (Figure 1).[5] This web-based module, launched in February 2018, was conceived and designed to provide information on and to visualise the cancer burden indicators. The application provides views across three main types of information: historical incidence and mortality indicators at registry level, national incidence and mortality predictions, and national survival estimates. It provides the means for comparing geographical patterns and temporal trends of incidence, mortality, and survival data of up to 58 different cancer sites. The database feeding the web application is dynamic, and is updated as new data become available. It currently hosts more than 34 million cancer cases submitted by approximately 150 European population-based CR in 34 European countries.

Since the ECIS web application provides information on geographical patterns and temporal trends of cancer burden indicators at national and/or regional level, it constitutes an

ECIS - European Cancer Information System
Measuring cancer burden and its time trends across Europe

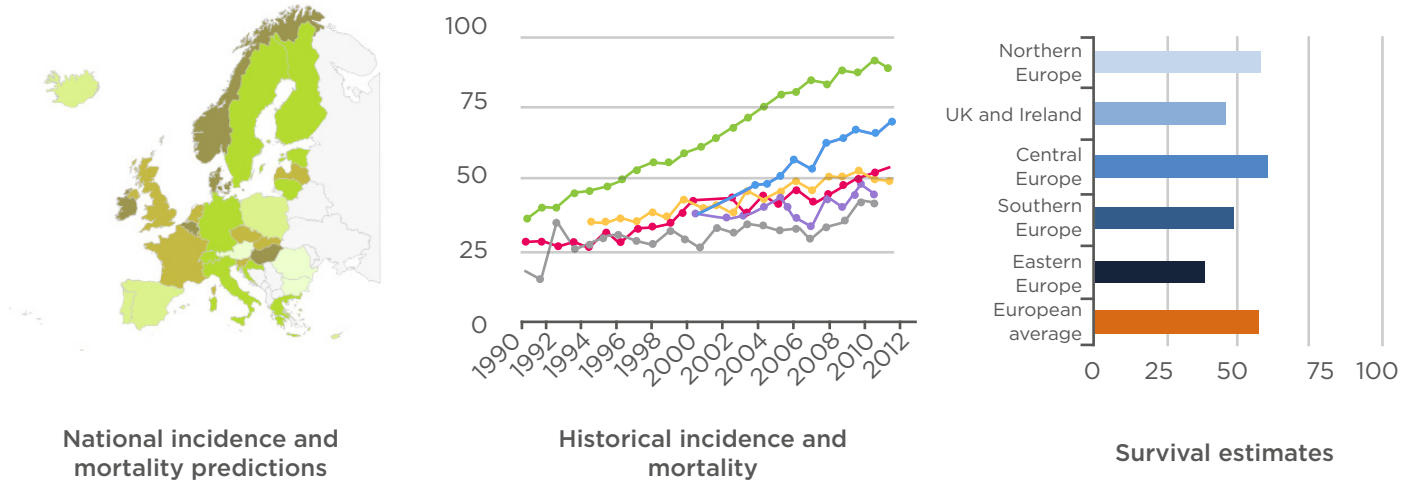

Figure 1: The three data modules of the ECIS web application.

Adapted from www.ecis.jrc.ec.europa.eu

important tool for promoting awareness of the cancer burden dynamics confronting Europe. ECIS is a major step forward as an information source for the citizens, as well as in assisting political decision making and supporting epidemiological research.

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Phase I Combination Dose-Finding and Phase II Expansion Cohorts of Lenvatinib with Etoposide and Ifosfamide in Patients Aged 2 to ≤25 Years with Relapsed or Refractory Osteosarcoma

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Disclosure: The authors have declared no conflicts of interest.

Keywords: Lenvatinib, osteosarcoma, vascular endothelial growth factor (VEGF).

Citation: EMJ Oncol. 2019;7(1):xx-xx. Abstract No: AR06.

BACKGROUND

Osteosarcoma is the most common primary bone tumour in adolescents and young adults, with no survival improvement in the last few decades. Lenvatinib (LEN) is a multikinase inhibitor of vascular endothelial growth factor (VEGF) receptors 1–3 and other targets. This study reports data from Phase Ib dose-finding and Phase II expansion cohorts of LEN with etoposide and ifosfamide chemotherapy in patients with relapsed or refractory osteosarcoma.[1] Single-agent safety and efficacy data were presented at the American Society of Clinical Oncology (ASCO) meeting in 2018.[2]

METHODS

Patients of the study were aged 2 to ≤25 years, with relapsed or refractory osteosarcoma and <2 prior VEGF-targeted therapies. The Phase Ib starting dose of LEN was 11 mg/m^2/day, ifosfamide 3000 mg/m^2, and etoposide 100 mg/m^2 daily for 3 days. On determination of the recommended Phase II dose (RPh2D) of LEN with chemotherapy, patients were enrolled into the Phase II expansion cohort. The primary endpoint of Phase Ib was upon RPh2D determination and Phase 2 endpoint was determined by 4 month progression-free survival (PFS-4).

RESULTS

In the Phase Ib dose-finding cohort (n=22), patients received LEN 11 mg/m^2 (n=7) and 14 mg/m^2 (n=15) with chemotherapy. Dose-limiting toxicities included Grade 4 thrombocytopenia (n=1; LEN 11 mg/m^2), Grade 4 thrombocytopenia and Grade 3 epistaxis (n=1; LEN 14 mg/m^2), Grade

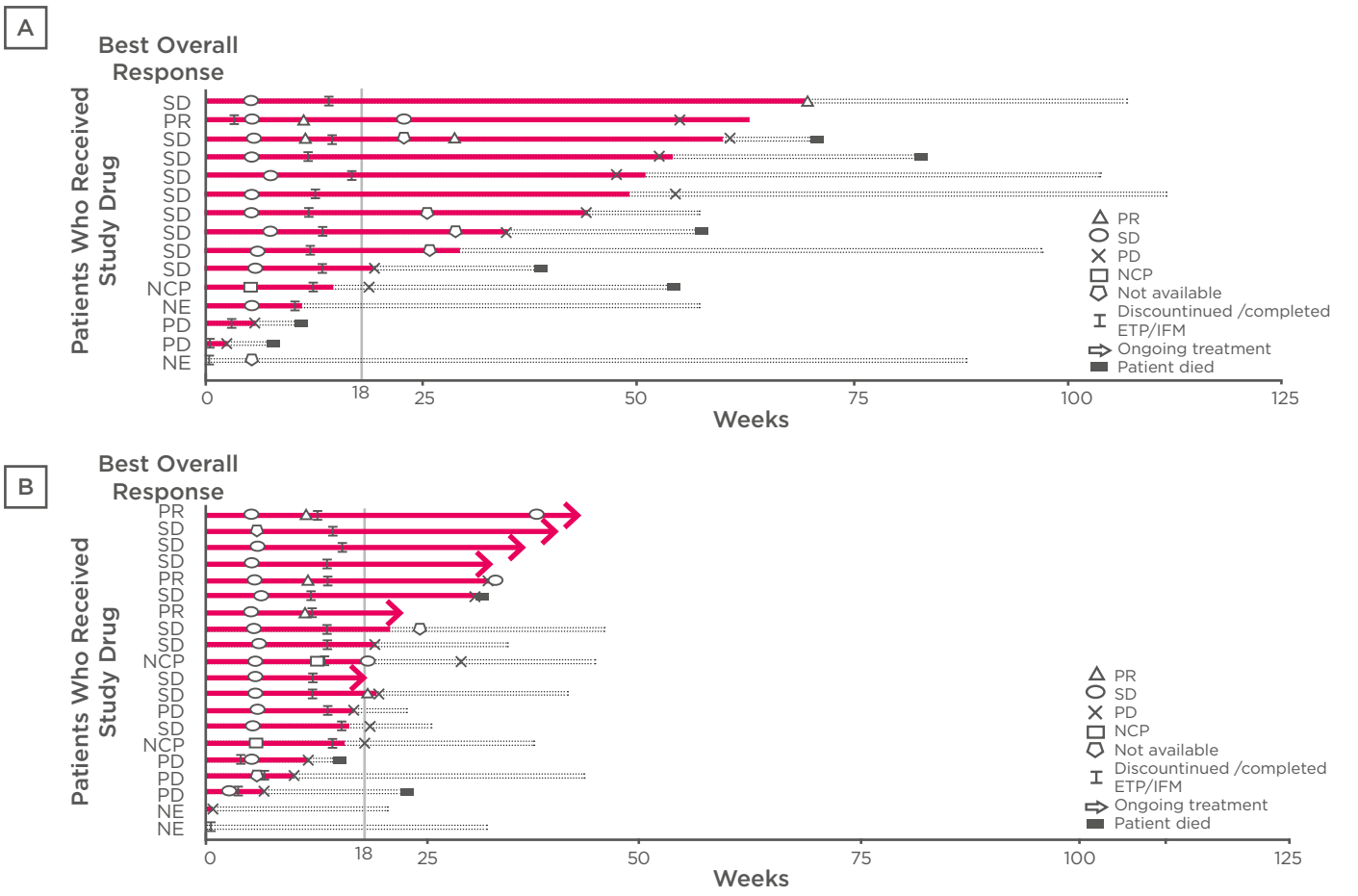

Figure 1: Duration of treatment, best overall response, and change of response over time (full analysis set: Lenvatinib [14 mg/m²] plus IFM plus ETP) for Phase Ib (A) and Phase II (B).

Each bar with solid line represents treatment duration, while the extended bars with the dashed lines represent the duration that the patient remained on the study after treatment discontinuation. A dose of 14 mg/m² of Lenvatinib had been planned.

ETP: etoposide; IFM: ifosfamide; NCP: non-complete response or progressive disease; NE: not evaluable; PD: progressive disease; PR: partial response; SD: stable disease.

2 oral dysesthesia, Grade 3 muscle spasm, and Grade 2 back pain (n=1; LEN 14 mg/m²). RPh2D was recorded as LEN 14 mg/m² with chemotherapy. In the expansion cohort (n=20), the median number of LEN cycles received was 4 (range: 1–7).

As reported in the database, the most frequent treatment-emergent adverse events (TEAE) were decreased platelet count or thrombocytopenia (50%/30%), neutropenia or neutrophil count decrease (45%/25%), anaemia (45%), nausea (40%), alanine aminotransferase level increase, diarrhoea, and white blood cell count decrease (30% each). The most frequent Grade ≥3 TEAE were neutropenia or neutrophil count decrease (45%/25%), platelet count decrease or thrombocytopenia (40%/20%), white blood cell count decrease (30%), and anaemia (25%). Most of these side effects were chemotherapy related. Pneumothorax was observed in the dose-finding cohort (n=6) and expansion cohort (n=1); two (dose-finding cohort) were Grade ≥3, and one was post-thoracotomy. In the dose-finding cohort, four patients discontinued treatment due to TEAE. There were no fatalities reported as a result of a treatment related TEAE.

In terms of efficacy, in the dose-finding combination cohort, 12 out of 18 evaluable patients (66.7%) achieved PFS-4 (Figure 1A). In the Phase II expansion cohort, 5 out of 8 evaluable patients (62.5%) achieved PFS-4 (Figure 1B).

CONCLUSION

The combination of RPh2D LEN (14 mg/m^2) with chemotherapy has a manageable safety profile with promising preliminary evidence of efficacy. In 2020, a randomised Phase II Trial (etoposide and ifosfamide) with or without LEN will take place (E7080-G000-230/ITCC-082) to evaluate the added value of LEN to second line chemotherapy in refractory or relapsed osteosarcoma.

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The Anticancer Power of the Immune System – New Perspectives for Patients with Triple-Negative Breast Cancer

EDITOR'S PICK

In an informative review by Rygiel, the powerful application of immunotherapy to tackle triple-negative breast cancer (TNBC) is given a thorough analysis. Compared to other breast cancer subtypes TNBC is associated with poor patient outcomes, meaning that comprehensive data pertaining to recent clinical trials and the new approaches being employed to tackle the condition is immensely valuable to clinicians and researchers. Here, new immunotherapy agents such as atezolizumab are discussed, and diagnostic considerations relevant to patients are presented in an engaging read.

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Disclosure: The author has declared no conflicts of interest.

Received: 08.07.19

Accepted: 25.09.19

Keywords: Checkpoint inhibitors, immunotherapy, programmed death-ligand 1 (PD-L1), programmed cell death protein 1 (PD-1), targeted therapy, triple-negative breast cancer (TNBC).

Citation: EMJ Oncol. 2019;7[1]:54-62.

Abstract

Triple-Negative Breast Cancer (TNBC) represents a heterogeneous disease that includes different subtypes and accounts for approximately 20% of all breast cancers (BC). TNBC is oestrogen receptor-negative, progesterone receptor-negative, and human epidermal growth factor receptor 2-negative. In addition, the androgen receptor is expressed in roughly 10–32% of TNBC cases. TNBC is characterised by worse outcomes, including higher risks of relapse and visceral crisis compared to other BC subtypes (especially during the first 2 years post BC diagnosis).

Programmed death-ligand 1 (PD-L1) is widely expressed on the surface of lymphocytes, monocytes, natural killer cells, macrophages, and some other cells. Moreover, PD-L1 expression has been explored in different types of cancer (e.g., malignant melanoma, non-small cell lung cancer, renal cell carcinoma, and colon cancer).

Due to limited treatment options for TNBC, there is an urgent need for the development of novel diagnostic and therapeutic strategies. To fulfil this unmet need, different approaches, including immunotherapy, have been investigated in clinical studies (with the goal of matching therapies with specific BC subtypes). This article discusses some diagnostic considerations relevant to patients

with TNBC (focussing on advanced or metastatic disease). It summarises the recent clinical trials, investigating novel targeted immunotherapeutic agents (e.g., pembrolizumab and atezolizumab) for TNBC, and highlights important implications for both research and clinical practice.

INTRODUCTION

Despite the impressive advances that have been made in cancer diagnosis and therapy, there still exist groups of patients who are not responding to standard anticancer treatments. In particular, triple-negative breast cancer (TNBC) represents a difficult-to-treat, heterogeneous disease that includes different subtypes and accounts for approximately 20% of all BC.[1] TNBC is oestrogen receptor (OR)-negative, progesterone receptor (PR)-negative, and human epidermal growth factor receptor 2 (HER2)-negative.[1] In addition, androgen receptor (AR) is expressed in roughly 10–32% of TNBC.[2] TNBC is characterised by worse outcomes, including higher risks of relapse and visceral crisis, compared to other BC subtypes (especially during the first 2 years post BC diagnosis).[3] Because treatment options for TNBC are very limited, there is an urgent need for the development of novel therapeutic options associated with reliable diagnostic tests. There is a growing interest in targeting the immune system as part of BC therapy.[4] According to the cancer immunoediting model, the immune system plays a dual role that consists of the host protection (via elimination of tumour cells) and the impact on the tumour (via editing its genome).[5] In this context, using immune checkpoint blockers can potentiate immunoediting. This, in turn, may contribute to 'shaping' the tumour and enforcing T-cell-dependent immunoselective efforts (via the immune checkpoint blockade).[5]

Programmed death-ligand 1 (PD-L1) is an Ig superfamily haplotype Type I transmembrane glycoprotein (related to apoptosis).[6,7] PD-L1 is widely expressed on the surface of lymphocytes, monocytes, natural killer (NK) cells, macrophages, and many other cells.[7] Moreover, PD-L1 expression has been explored in different types of cancer, including malignant melanoma, non-small cell lung cancer, renal cell carcinoma, colon cancer, and oesophageal cancer.[7] Similarly, programmed cell death protein 1 (PD-1), which is an inhibitory immune checkpoint that limits T-cell effector functions within tissues, is expressed on the surfaces of immune effector cells (such as T cells, B cells, NK cells, dendritic cells [DC], and many tumour infiltrating lymphocytes [TIL]).[7] Recently, some novel immunomodulatory agents, including immune checkpoints inhibitors, have shown promising effects in subgroups of women with advanced or metastatic TNBC.[8] For instance, increased PD-L1 expression on the surface of TNBC cells provides the target for such immunotherapeutic strategies. In particular, the PD-L1 inhibitor, atezolizumab, and the PD-1 inhibitor, pembrolizumab, have revealed beneficial results in recent clinical trials (Table 1).[8]

This mini review presents some novel diagnostic considerations related to patients with TNBC, focussing on advanced or metastatic disease. It summarises the main clinical trials leading to approval of immunotherapy (e.g., targeting the PD-1/PD-L1 pathway) for TNBC, and highlights important implications for further research and clinical oncology practice.

CANCER IMMUNOEDITING, IMMUNOLOGICALLY-RESPONSIVE, AND IMMUNOLOGICALLY-IGNORANT TUMOURS

Multiple molecular changes, which occur as a result of malignant tumour progression, should facilitate the distinction between cancer cells and healthy cells. Consequently, tumour cells should be recognised as foreign by the immune system, and subsequently destroyed. Unfortunately, tumours are seldom rejected spontaneously because of their capability to maintain an immunosuppressive microenvironment.[18] In fact, the interplay between cancer cells and immune system cells (within the tumour microenvironment) creates the possibility for neoplastic cells to escape from immune surveillance.[18] Based on the cancer immunoediting concept, the immune system (via interactions between tumour and host) recognises tumour-specific antigens, protects the host (by elimination of tumour cells), and 'shapes' the developing tumour (via editing the cancer genome).[18] In this way, the tumour immunogenicity is being reduced.[18]

Table 1. Recent clinical trials on immune checkpoint inhibitors in patients with locally advanced or metastatic triple-negative breast cancer.

Name of the checkpoint inhibitor	Trial name, identifier, Phase	Aims of the trial	Clinical relevance of the trial	Author, year
Pembrolizumab anti-PD-1 antibody	KEYNOTE-012 NCT01848834 Ib	Evaluation of pembrolizumab (single-agent) in patients with PD-L1-positive TNBC, gastric cancer, urothelial cancer, and head and neck cancer.	Pembrolizumab has shown an acceptable safety profile in patients with advanced or metastatic TNBC.	Nanda et al., 2016[9]
Pembrolizumab	KEYNOTE-086 NCT02447003 II	Evaluation of pembrolizumab as first-line therapy for patients with PD-L1-positive metastatic TNBC.	Pembrolizumab monotherapy had a manageable safety profile and durable anti-tumour activity as first-line therapy for patients with PD-L1-positive metastatic TNBC.	Adams et al., 2019[10]
Pembrolizumab	KEYNOTE-355 NCT02819518 III	Study of pembroizumab plus CHT vs. placebo plus CHT (one of the regimens: paclitaxel or nab-paclitaxel or gemcitabine/carboplatin) for previously untreated locally recurrent inoperable or metastatic TNBC.	Patient stratification factors: CHT used in the study (taxane vs gemcitabine/carboplatin), tumour PD-L1 expression (+/-), and prior therapy with same-class agent in the (neo) adjuvant setting; primary end points: safety in Part 1, PFS and OS in Part 2; secondary end points: ORR and duration of response (ongoing).	Cortés et al., 2018[11]
Pembrolizumab	KEYNOTE-119 NCT02555657 III	Comparison of pembrolizumab alone with single-agent CHT (per investigator's choice) in patients with metastatic or locally advanced TNBC.	Pembrolizumab monotherapy vs single agent CHT for advanced or metastatic TNBC (ongoing).	Clinical Trials.gov 2018[12]
Pembrolizumab	KEYNOTE-522 NCT03036488 III	Study of pembrolizumab plus CHT vs placebo plus CHT as neoadjuvant therapy followed by pembrolizumab vs. placebo as adjuvant therapy for TNBC.	Primary end points are pCR rates; secondary end points are safety, OS, and pCR rate in all patients; and OS, EFS, and pCR rate in women with PD-L1–positive tumours (CPS≥1). Adult patients with previously untreated, locally advanced, nonmetastatic TNBC are eligible (ongoing).	Schmid et al., 2018[13]

Table 1. continued

Durvalumab anti-PD-L1 antibody	GeparNuevo II	Neoadjuvant therapy in patients with early-stage TNBC investigating the role of durvalumab, in addition to standard CHT with nab-paclitaxel followed by epirubicin plus cyclophosphamide.	Immunomonitoring of TNBC patients undergoing neoadjuvant therapy (GBCG89); it is expected that possible biomarkers for the treatment of TNBC patients will be identified (leading to better patient selection for CHT/ immune combination therapy) (ongoing).	Seliger, 2018[14]
Atezolizumab anti-PD-L1 antibody	IMpassion031 III	Study comparing neoadjuvant atezolizumab vs placebo in combination with nab-paclitaxel-CHT in early TNBC.	Primary end-point is pCR; secondary end-points are pCR according to PD-L1 status, patient-reported outcomes, EFS, and OS; tumour samples will be assessed for biomarkers associated with response and immune escape (ongoing).	Mittendorf et al., 2018[15]
Atezolizumab	IMpassion130 NCT02425891 III	Atezolizumab with nab-paclitaxel vs placebo with nab-paclitaxel for patients with previously untreated advanced or metastatic TNBC.	PFS and OS were improved in PD-L1–positive patients; the PD-L1 expression in immune cells is a predictor of response; in PD-L1–negative patients there was no therapeutic effect of atezolizumab and nab-paclitaxel	Schmid et al., 2018[16]
Nivolumab anti-PD-1 antibody	TONIC NCT02499367 II	Study of strategies stimulating the anticancer immune responses (by induction treatment with irradiation or low dose CHT) to make the tumour microenvironment more susceptible to nivolumab in metastatic TNBC.	Short-term induction with irradiation or low dose CHT (doxorubicin, cyclophosphamide, or cisplatin) before nivolumab is feasible in metastatic TNBC.	Kok et al., 2018[17]

CHT: chemotherapy; CPS: combined positive score; EFS: event-free survival; IHC: immunohistochemistry; ORR: overall response rate; OS: overall survival; pCR: pathologic complete response; PD-1: programmed cell death protein-1; PD-L1: programmed death ligand 1; PFS: progression-free survival; TNBC: triple-negative breast cancer.

At present, there is a need to explore the role of cancer immunoediting in the context of anticancer immunotherapy.[19] For instance, anticancer immunotherapies with checkpoint inhibitors, such as anticytotoxic T lymphocyte-associated antigen 4 or anti-PD-1/-PD-L1 antibodies, have revealed some positive clinical responses.[19] However, one of the greatest challenges is intrinsic resistance to immunotherapy and the development of resistant disease after therapy, i.e., an acquired resistance to immunotherapy.[19] In an effort to address these patterns, anticancer immunotherapy, with the use of modern biomarkers, has emerged as a novel treatment modality for various, difficult-to-treat malignancies.[19,20]

In general, malignancies can be classified as immunologically-responsive or immunologically-ignorant.[20] Immunologically-ignorant tumours are characterised by low mutation load, immune tolerance against self-antigens, and absence of infiltrating T cells.[12] In contrast, immunologically-responsive tumours are characterised by the presence of numerous infiltrating T cells, which illustrate intrinsic T-cell immune-inhibition and extrinsic tumour-related T-cell immuno-suppression.[22] The process of T-cell immune-inhibition is mediated via activation of immune checkpoint molecules (e.g., cytotoxic T lymphocyte-associated antigen 4, PD-1, T-cell immunoglobulin mucin-3, and lymphocyte-activation gene 3).[19,21] This article will focus on the PD-1/PD-L1 checkpoints.

IMMUNE CHECKPOINTS - THEIR PHYSIOLOGICAL ROLE AND THERAPEUTIC POTENTIAL IN PATIENTS WITH CANCER

Physiologically, immune checkpoint molecules are 'in charge' of preventing tissue damage that occurs during infections and autoimmunological processes. Immune checkpoints are inhibitory receptors, which are mostly expressed on the surfaces of T cells and tumour cells where they mediate the interactions between these cells.[23] In an adaptive immune resistance mechanism, the involvement of immune checkpoints on T cells by tumour cells suppresses the cytotoxic ability of T cells.[24] This allows tumour cells to escape cytotoxicity and protects cancer from immune system attacks.[24]

T cell immune-inhibition decreases activity of cytotoxic T lymphocytes and reduces the recruitment of anti-inflammatory cells, regulatory T cells, and myeloid-derived suppressor cells.[21,24] When PD-1 receptors on T lymphocytes are activated and bound to their relevant ligands PD-L1 and PD-L2, these immune checkpoints inhibit T-cell functions. In this way, the PD-1/PD-L1 axis is responsible for regulation of T-cell activation and prevention of tissue damage. On the other hand, however, the PD-1/PD-L1 axis enables tumour cells to evade immune surveillance.[21,24]

PD-L1 EXPRESSION ON THE TUMOUR CELLS AND TUMOUR INFILTRATING LYMPHOCYTES – RELATIONS WITH THERAPEUTIC EFFECTS

According to the concept supported by the results of the KEYNOTE-001 trial,[12] related to immunotherapy for patients with cancer, it has been suggested that elevated tumour cell expression of PD-L1 correlates with immune system evasion. This, in turn, can lead to a worse prognosis among patients with malignancies treated with checkpoint inhibitors.[25]

However, based on a study of patients with cancer receiving therapy with anti-PD-L1 agents, in which two times as many patients with low or no PD-L1 expression on the tumour cells had beneficial clinical outcomes compared to the ones who had tumours with PD-L1 overexpression, it was revealed that this relation differed in various types of cancers.[18,19]

Additionally, according to a recent, large meta-analysis, which has addressed the expression of PD-L1 and prognosis in patients with BC, it has been revealed that PD-L1 positivity was ranging from 21 to 56%.[26] Furthermore, it should be noted that in the majority of PD-L1-positive BC, PD-L1 expression was focal and limited to a small proportion of cancer cells, rather than diffuse.[27]

To date, TNBC has been viewed as 'immunogenic' (e.g., in terms of PD-L1 expression in both tumour and inflammatory cells, such as TIL).[28] As a consequence, anti-PD-1/PD-L1-targeted therapies can be added to the TNBC treatment arsenal.[28,29] In fact, TNBC has elevated PD-L1 expression, mostly in inflammatory (immune) cells and in some malignant cells.[27,29] TIL can increase during both

chemotherapy (CHT) and radiotherapy, and this may be caused by increased activation of CD8+ T cells and IFN-gamma secretion (during CHT or RT), which can stimulate PD-L1 expression.[30] Elevated levels of TIL have been associated with improved disease-free survival and overall survival rates among TNBC patients.[30] In addition, the presence of TIL in the breast tumour microenvironment may (to some degree) predict responses to neoadjuvant and adjuvant CHT.[30] For instance, elevated numbers of TIL correlate with increased pathological complete responses (pCR) in patients with TNBC.[30] Therefore, TIL play the role of prognostic and predictive markers of response to anticancer therapies.[30]

PD-1/PD-L1 AXIS AND BIOMARKERS OF RESPONSE TO PD-1/PD-L1 INHIBITION

PD-L1 overexpression in tumour cells may be considered a prognostic biomarker, but not a predictive biomarker due to different factors.[25] For instance, PD-L1 expression may be influenced by TIL that produce IFN-gamma which, in turn, contributes to more beneficial clinical outcomes.[25] Despite using various immunohistochemistry (IHC) staining methods, there is still no standard procedure for evaluation of PD-L1 expression.[25] This is partially because of the fact that the PD-L1 heterogeneity reflects a dynamic process, in which a tumour may not express PD-L1 at baseline. It should be highlighted that TNBC has a higher level of PD-L1 expression, thus a blockade of PD-L1 with the use of novel immune checkpoint inhibitors can activate tumour-specific T-cell responses, leading to enhanced anti-tumour activity and better outcomes for this group of patients.[31]

An innovative application of the immune checkpoint inhibitors against either PD-1 or its PD-L1 have reshaped the therapeutic landscape of many difficult-to-treat malignancies, including TNBC.[18,19,32] The interplay between PD-1 on T-cells and its ligands PD-L1 and PD-L2 on malignant cells causes T-cell exhaustion and leads to conversion of T effector cells to immunosuppressive T regulatory cells.[32] In this scenario, the immune checkpoint inhibitors (acting against PD-1 or PD-L1) block the suppressor PD-1/PD-L1 axis. This leads to the reactivation of cytotoxic T effector cells and invigoration of the anticancer power of the immune system.[32]

IMMUNOLOGIC CONSIDERATIONS IN METASTATIC TNBC

It should be noted that metastatic BC represent microenvironmental systems, in which cell proliferation and apoptosis often coexist with immune system cell infiltration. Apoptotic tumour cells undergo phagocytosis, and tumour-specific antigens are expressed on the major histocompatibility complex molecules by tumour-infiltrating antigen presenting cells (APC). Subsequently, APC can activate antigen-specific cytotoxic T lymphocyte responses.

Under these circumstances, metastatic TNBC, which is positive for PD-L1, responds to a combination therapy with monoclonal anti-PD-L1 antibody (e.g., atezolizumab) and CHT (e.g., nab-paclitaxel).[16] In this context, an agent such a nab-paclitaxel increases expression of tumour-specific antigens and invokes apoptosis, contributing to the antigen presentation via APC. In this way, suppressive signal inactivating T cells is stopped because the immune checkpoint inhibitor (e.g., atezolizumab) blocks the interaction between PD-1 and its ligands (PD-L1/2) to reverse T-cell suppression.[32]

INSIGHTS INTO THE IMPASSION130 TRIAL

The promising results of the randomised clinical trial (RCT) IMpassion130 have led to approval of atezolizumab (an anti-PD-L1 agent) in combination with CHT (using nanoparticle albumin-bound [nab] paclitaxel) for the therapy of patients with unresectable, locally advanced, or metastatic TNBC (Table 1)[16]. This approval was based on the Phase III RCT (atezolizumab plus nab-paclitaxel versus placebo plus nab-paclitaxel), involving >900 women with TNBC (with no previous treatment for the metastatic BC). The reason for using a combination of the checkpoint inhibitor with taxane-based CHT (which blocks mitosis) was that this therapy can increase the tumour-antigen release and augment anti-tumour responses to the immune checkpoint inhibition.[33] It should be noted that in the IMpassion130 trial, prior to applying the atezolizumab therapy, tumour samples were evaluated by IHC for the presence of PD-L1 expression (using SP142 clone, Ventana, Roche,

Switzerland).[16] PD-L1 expression was assessed via the presence of tumour-infiltrating (immune) cells, using "a percentage of tumour area" <1% (meaning PD-L1 negative status) or ≥1% (meaning PD-L1 positive status).[16] The IMpassion130 trial has shown that the patients whose cancers were positive for PD-L1 (roughly 41%) and received atezolizumab had better outcomes compared to the ones treated with nab-paclitaxel only (i.e., median progression-free survival was 7.2 months in the atezolizumab–nab-paclitaxel arm, compared to 5.5 months in the placebo-nab-paclitaxel arm).[16] In the PD-L1-positive subgroup, the response rate was approximately 59% in the atezolizumab-nab-paclitaxel group, compared to approximately 43% in the placebo–nab-paclitaxel group.[16] It should be pointed out that 10% of the patients in the atezolizumab–nab-paclitaxel group achieved a complete response, compared to only 1% of the ones in the placebo–nab-paclitaxel group.[16] In addition, the atezolizumab plus nab-paclitaxel combination therapy arm has revealed a relatively good safety profile (i.e., the most typical adverse effects included hair loss, nausea, vomiting, diarrhoea, constipation, poor appetite, fatigue, tingling or numbness in the hands and feet, anaemia, cough, headache, and neutropenia).[16]

PD-L1 expression in both cancer cells and immune cells (as detected by IHC) represents a predictive biomarker according to the IMpassion130 trial.[16] In addition, diagnostic antibodies have been validated as companion or complementary diagnostics. In accordance with the U.S. Food and Drug Administration (FDA) definition, companion diagnostics is a medical device that provides information that is essential for the safe and effective use of a corresponding drug or biological product. Similarly, complementary diagnostics is a test that aids in the benefit–risk decision-making about the use of the therapeutic product, in which the difference in benefit–risk proportion is clinically meaningful.[34] It should be noted that SP142 (Ventana) represents the companion/complementary diagnostics not only for some subtypes of BC, but also for non-small cell lung cancer and bladder cancer.[34] In fact, the recent approval of atezolizumab for the treatment of TNBC is applicable only to patients in whom BC express PD-L1, based on the Ventana diagnostic antibody SP142 test.[16,34]

FOCUS ON PD-L1 EXPRESSION ON THE IMMUNE CELLS INSTEAD OF THE TUMOUR CELLS

It should be noted that in the IMpassion130 trial, PD-L1 staining was focussed on PD-L1 expression on the immune cells, instead of the tumour cells (contrary to other types of cancer, such as the lung cancer).[16] For instance, in the IMpassion130 trial 41% of patients were classified as PD-L1-positive, based on immune cells staining (at a cut-off of 1%).[16] In addition, in this trial only 9% of patients were classified as PD-L1-positive on BC tumour cells, and most of them were also PD-L1-positive on the immune-cells. In fact, there may by some prognostic value (or a small difference in outcomes) in patients with PD-L1-positive expression, based on the immune versus tumour cell staining.[16] In contrast, only approximately 2% of patients were PD-L1 positive on the tumour cells and PD-L1-negative on the immune cells. In addition, almost 60% of patients who were PD-L1 negative on the immune cells did not appear to derive substantial benefits from atezolizumab.[16] Therefore, routine testing for PD-L1 status, among newly diagnosed patients with metastatic and locally advanced TNBC, should be merited to determine whether or not they can derive benefits from atezolizumab and nab-paclitaxel combination treatment.[16] At this point, it is important to explain some key differences in the biology of PD-L1-positive and PD-L1-negative tumours. Moreover, making the PD-L1-negative tumours more immunogenic (e.g., via adding another agent, which may possibly alter their immunogenicity) can hopefully offer some therapeutic benefits.

FURTHER RESEARCH DIRECTIONS FOR IMMUNE CHECKPOINT INHIBITORS IN LOCALLY ADVANCED AND METASTATIC TRIPLE-NEGATIVE BREAST CANCER

In addition to the IMpassion130 trial, some important lessons have been learned from recent or ongoing clinical trials on immune checkpoint inhibitors in advanced or metastatic TNBC (Table 1).[9-17] Continuous efforts are still necessary to optimise the therapy in the PD-L1-positive patients, and to design innovative approaches for the PD-L1-negative patients with TNBC. Due to remarkable

progress in molecular characteristics of TNBC, not only immune checkpoint inhibitors, but also some other modern therapeutics, including poly ADP-ribose polymerase-1 inhibitors, tyrosine kinase inhibitors, androgen receptor inhibitors, and antibody-drug conjugates, are going to be explored in depth for this highly aggressive subtype of BC.[35]

It should be highlighted that it is essential to properly identify patients who may favourably respond to therapies with PD-1/PD-L1 checkpoint inhibitors. Moreover, in the dynamic interplay of the cancer cells with different immune cells (e.g., T lymphocytes, B lymphocytes, and APC), reliable biomarkers are needed to precisely predict the effects of the PD-1/PD-L1 checkpoint inhibitors among patients with TNBC. In addition to the PD-1/PD-L1 status, such biomarkers may include tumour mutational burden/load, microsatellite instability status, and the number of TIL.[8,19,21,30,32,35]

Further investigation in TNBC should also involve predictive and prognostic biomarkers for proper stratification of patients, who would be the most appropriate candidates for immune checkpoint inhibitor therapies.[14,15,36] In addition, the optimal timing of administration and the best combination approaches (e.g., CHT, targeted therapy, and radiotherapy; administered concomitantly or sequentially) represent the main research questions that need to be addressed.[35,36] Moreover, the treatment responses, survival outcomes, and safety issues, in monotherapy and in combination therapy should be investigated long term, in large-scale RCT. For instance, the anti-PD-1 and anti-PD-L1 monoclonal antibodies (e.g., pembrolizumab, durvalumab, atezolizumab, and nivolumab) are currently being tested in clinical trials among women with locally advanced or metastatic TNBC (in neoadjuvant or adjuvant settings) (Table 1).[11-15,17] Furthermore, in the early TNBC setting, an assessment of the benefits of possible adding the immune checkpoint inhibitors to the neoadjuvant CHT is going to be explored.[14,15,36] Simultaneously, studying communication networks between cancer cells and immune cells in the tumour microenvironment, as well as developing multi-modal management plans aimed at inducing anti-tumour immune responses, may hopefully improve clinical outcomes among patients with TNBC.[37,38]

CONCLUSION

Advances in cancer immunotherapy highlight the necessity of continuous learning about cancer immunology and the interactions of immune cells and tumour cells within the malignant tumour and its microenvironment. Novel immunotherapy strategies magnify the immune system actions and evoke durable tumour-specific immune memory. Consequently, some monoclonal antibodies that mediate the immune checkpoint receptors have provided promising improvements for patients with TNBC (e.g., in the metastatic setting).

Immune checkpoint blockade as monotherapy or combination therapy (e.g., atezolizumab and nab-paclitaxel) demonstrated some encouraging results as first-line therapy for metastatic TNBC (e.g., improvements in PFS, compared to CHT alone). The recent FDA approval of atezolizumab, a selective immune checkpoint inhibitor targeting PD-L1, plus nab-paclitaxel for the treatment of patients with PD-L1-positive, unresectable, locally advanced, or metastatic TNBC augments the therapeutic armamentarium for such a challenging BC subtype.

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Epithelial Myoepithelial Carcinoma of the Hard Palate: A Case Report with a Review of the Literature

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Disclosure: The authors have declared no conflicts of interest.

Received: 10.11.18

Accepted: 14.03.19

Keywords: Case report, epithelial myoepithelial carcinoma (EMC), hard palate.

Citation: EMJ Oncol. 2019;7[1]:63-67.

Abstract

Background: Epithelial myoepithelial carcinoma (EMC) is a rare biphasic tumour of the salivary gland with two cell types of inner ductal cells and outer layer of clear cells. In the literature, there are only a few reports of EMC originating from the hard palate.

Case report: A 58-year-old female presented to the authors' institution with partially submucosal lesion in the posterior aspect of the hard palate on the left side for 1 month. Biopsy was suggestive of a multinodular tumour with round to oval cells and a moderate number of pale eosinophilic to clear cytoplasm and round to oval, centrally to eccentrically placed, mildly pleomorphic vesicular nuclei suggestive of EMC of the hard palate. Immunohistochemically, cytokeratin (CK 5/6) showed strong cytoplasmic positivity highlighting the luminal epithelial cells. The myoepithelial cells showed strong nuclear positivity for p63 and cytoplasmic positivity for calponin. The patient underwent surgical resection of the tumour with a local flap cover and split skin graft and all the margins were negative in the final histopathological examination with erosion of the underlying bone. The patient was kept under observation and has been free of the disease for the past 12 months.

Conclusion: Diagnosis of EMC is rare and is to be kept as a differential diagnosis during the evaluation of minor salivary gland tumours of palate.

BACKGROUND

Epithelial myoepithelial carcinoma (EMC) was initially described by Donath et al.[1] and was previously referred to with various terminologies such as adeno-myoepithelioma, clear cell adenoma, or carcinoma. EMC is a rare biphasic tumour of the salivary gland with two cell types: an inner layer of duct lining cells and an outer layer of clear cells, which typically form double-layered duct-like structures.[2] The tumour has a relatively low incidence, accounting for <1% of malignant salivary gland tumours.[3,4] This tumour arises most frequently in the parotid gland (80%), but lesions have also been reported in the submandibular glands (10%) and minor salivary glands (1%).[5] In the minor glands of the oral mucosa, EMC represents approximately 5% of all salivary gland tumours.[6] Only a few case reports of EMC originating from the hard palate are present in the literature.[7-17] The authors herewith report this case for its rarity of the tumour in the hard palate.

CASE REPORT

A 58-year-old female presented to the oncology outpatient department with the complaint of ulcerative lesion in the upper palate of 1-month duration which was progressively increasing in size and was associated with occasional pain. Examination revealed a partially ulcerated submucosal lesion in the posterior aspect of the left side of the hard palate, 15 mm in front of the hard palate–soft palate junction, and not extending to the midline (Figure 1).

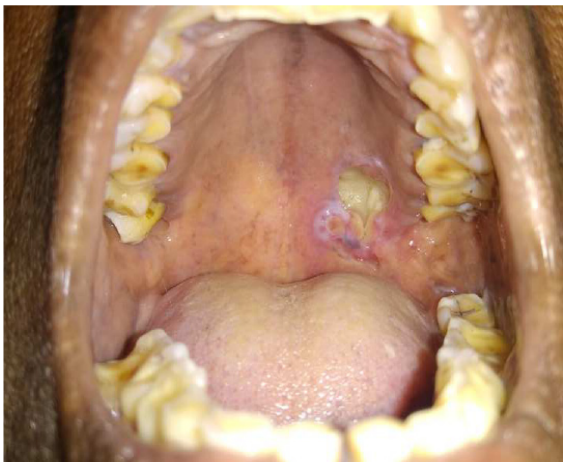

Figure 1: Clinical picture showing submucosal partially ulcerative lesion in the posterior aspect of the left side of the hard palate.

There were no significant palpable neck nodes. CT imaging identified thickening of the mucosa with underlying bone erosion, and there were no nodal metastases. The chest roentgenogram was normal.

Intraoral biopsy was performed, and the histopathological sections showed multinodular tumour with tumour cells arranged in an organoid fashion. The tumour was made up of round to oval cells with a moderate amount of pale eosinophilic to clear cytoplasm and round to oval, centrally to eccentrically placed, mildly pleomorphic vesicular nuclei, with few showing small prominent nucleoli. Focally luminal spaces lined by flattened cells were also noted, which were suggestive of EMC of the hard palate (Figures 2a and 2b). A diagnosis of EMC with predominance of myoepithelial clear cells was suggested. Immunohistochemistry was carried out to rule out other clear cell tumours of the salivary gland. Immunohistochemically, cytokeratin (CK 5/6) showed strong cytoplasmic positivity highlighting the luminal epithelial cells. The myoepithelial cells showed strong nuclear positivity for p63 and cytoplasmic positivity for calponin; however, staining for smooth muscle actin was negative (Figures 3a and 3b). Thus, the biphasic nature of the tumour was confirmed, and a diagnosis of EMC was established.

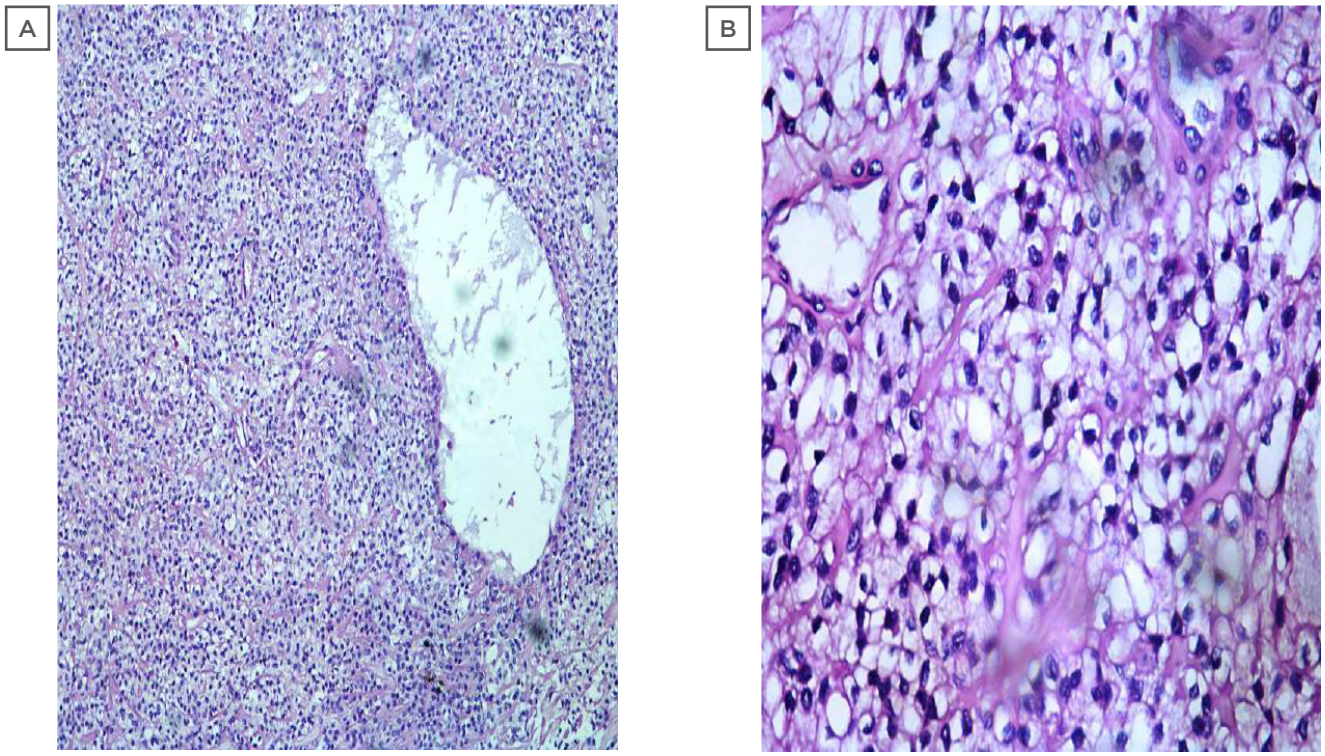

Figure 2: (A) photomicrograph showing epithelial component featuring duct like structures surrounded by clear myoepithelial cells (100X; haemotoxylin and eosin stain). (B) photomicrograph showing epithelial component featuring duct like structures lined by a single layer of cuboidal epithelium surrounded by clear myoepithelial cells.

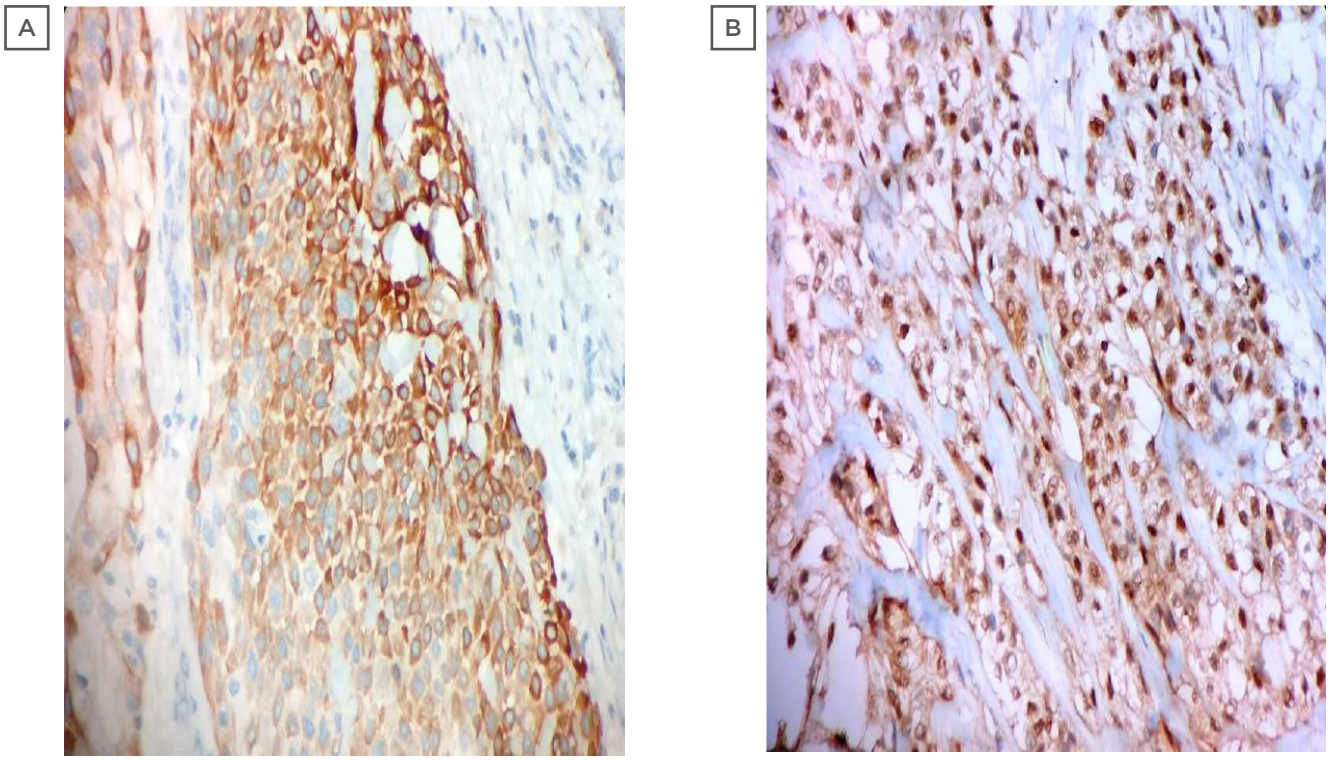

Figure 3: (A) photomicrograph showing the immunohistochemistry of strong cytoplasmic positivity of cytokeratin (CK 5/6) (400X; haemotoxylin and eosin stain). (B) photomicrograph showing the immunohistochemistry of strong nuclear positivity of P63 (400X, haematoxylin and eosin stain).

The patient underwent surgical resection of the tumour through modified Weber–Ferguson incision and partial maxillectomy was conducted with 10 mm gross margins, with the frozen section of the margins being negative (Figure 2a). The reconstruction of the tumour was performed with local flap cover and split skin graft. The postoperative course of the patient was normal. The final histopathological report suggested that the tumour, with a size of 30x15 mm, and the microscopic and immunohistochemistry features were the same as the preoperative biopsy. All the margins were free of tumour and the bone was eroded by the tumour. After discussion in the multispeciality tumour board clinic, the patient was given the option of observation or adjuvant radiation and the patient opted for observation. The patient has been free of the disease for the past 12 months.

DISCUSSION

EMC was defined as a solitary pathological diagnosis in 1991 by the World Health Organization (WHO).[18] The mean age of EMC patients is approximately 60 years and it affects females at a ratio of 1.5:1.0 with a mean tumour size of approximately 29 mm.[19] EMC is a low-grade malignancy, and high-grade or dedifferentiated EMC cases have rarely been reported. Some morphologically low-grade myoepithelial carcinomas behave aggressively. EMC is a malignant biphasic salivary-type tumour and the diagnosis is based on conventional light microscopy, confirmed by immunohistochemical and ultrastructural investigation. Histopathologically, the tumour is characterised by well-defined tubules with two cell types: an outer layer of myoepithelial cells with clear cytoplasm surrounding an inner lining of eosinophilic cuboidal epithelial cells resembling intercalated ducts.[20] In an observation made by Aydil et al.,[21] the most common malignancies in the hard palate are minor salivary gland tumours (60.6%), followed by benign mesenchymal tumours (15.2%), squamous cell carcinoma (12.1%), malignant melanomas (6.1%), lymphomas (3.0%), and sarcomas (3.0%).

Immunohistochemical diagnosis involves various criteria and studies have commonly reported positivity for epithelial markers including CK 7, 14, and 5/6; S100 protein; endothelial membrane antigen; and smooth muscle actin. Calponin and glial fibrillary acidic protein have also been reported to be sensitive markers of myoepithelial differentiation in salivary lesions. Furthermore, p63 has also recently become a widely-used marker for abluminal cells, in both basal and myoepithelial, showing nuclear immunoreactivity.[20] The tumour in this case was positive for p63 and calponin which helped in the conformation of the diagnosis. Recent molecular studies with PLAG1 and HMGA2 cellular rearrangements showed that 80% of EMCA arise from pleomorphic adenoma.[22]

EMC of the minor salivary glands are very rare and only a few cases have been reported with palatal origin.[7-17] Most of the reported cases had painless and well-circumscribed masses associated with surface ulceration. There is no consensus as to the treatment of minor salivary gland EMC as the number of patients is too small to allow for controlled treatment trials. Tumour resection with negative surgical margin is the primary modality of the treatment. The role of adjuvant chemotherapy in EMC is not well documented. Seethala et al.[19] found that the recurrence rate of EMC was 36.3%, and survival rates were 93.5% and 81.8% for 5 and 10 years, respectively.

CONCLUSION

In conclusion, diagnosis of EMC is rare and is to be borne in mind as a differential diagnosis during the evaluation of minor salivary gland tumours of the palate. The difficulty in establishing the pathological diagnosis implies the need for experienced pathologists and knowledge of immunohistochemical evaluation.

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PARP Inhibitors as a Novel Treatment Strategy for Patients with *BRCA*-Mutated Metastatic Breast Cancer

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Disclosure: The author has declared no conflicts of interest.

Received: 29.05.2019

Accepted: 02.07.2019

Keywords: Breast cancer (BC), germline *BRCA*-mutation (g*BRCA*m), homologous recombination deficiency (HRD), olaparib, poly(ADP-ribose) polymerase (PARP) inhibitors.

Citation: EMJ Oncol. 2019;7[1]:68-76.

Abstract

Inhibitors of poly(ADP-ribose) polymerase (PARP), such as olaparib and talazoparib, have recently been approved as therapies for *BRCA*-mutated human epidermal growth factor receptor 2 (HER2)-negative metastatic breast cancer (BC). In addition, olaparib, as well as rucaparib and niraparib, have received approval for treatment of patients with *BRCA*-mutated or platinum-sensitive recurrent ovarian cancer.

The treatment efficacy of PARP inhibitors is higher in case of malignancies that harbour deleterious germline or somatic *BRCA* mutations compared to *BRCA* wild-type tumours. Consequently, *BRCA* mutations or intrinsic tumour sensitivity to platinum therapy are considered indicators of impaired ability to repair DNA double-strand breaks via homologous recombination.

However, not all *BRCA*-mutated cancer patients benefit from PARP inhibitors. In contrast, for some patients with wild-type *BRCA* or platinum-resistant tumours, the PARP inhibitors may still offer some therapeutic advantages. Therefore, there is a need to determine additional biomarkers to more precisely select patients without deleterious *BRCA* mutations, who may be eligible for treatment with PARP inhibitors.

The main objective of this mini-review is to present the main mechanisms of action of PARP inhibitors and briefly summarise the clinical trials leading to their approval in treatment of *BRCA*-mutated, HER2-negative metastatic BC. In addition, this article discusses the efficacy, safety, and resistance to PARP inhibitors in women with metastatic BC.

INTRODUCTION

Physiologically, cells are equipped with DNA damage repair systems that can correct various errors. However, congenital defects in DNA repair mechanisms may cause accumulation of DNA mutations and elevated risk of malignancy. Certain genes that are involved in DNA damage repair pathways play a key role in neoplastic development. For instance, an elevated susceptibility to inherited breast cancer (BC)

and ovarian cancer (OC) has been reported in women harbouring germline mutations of *BRCA1* and *BRCA2* (both tumour suppressor genes).[1,2] Despite their structural differences, these genes perform similar cellular actions and are instrumental for genome protection.[1,2] The *BRCA1* gene is located on the long arm of chromosome 17, and *BRCA2* gene on the long arm of chromosome 13.[3] Currently, >2,000 mutations have been detected in both *BRCA* genes (e.g., deletions, insertions, or duplications) that result in various aberrant transcriptional outcomes (e.g., missense, nonsense, silent, and splice-site).[3] From a clinical point of view, *BRCA* changes that augment cancer susceptibility have been recognised as deleterious mutations, and usually result in nonfunctional proteins.[3] As a consequence, such mutations interfere with the repair of the damaged DNA. For instance, a large study on *BRCA1* or *BRCA2* mutation carriers has revealed a significant cumulative risk of BC and OC, in regard to both the *gBRCA1m* mutation (72% for BC and 44% for OC) and *gBRCA2m* mutation (69% for BC and 17% for OC).[4] In contrast, in the general population of women the cumulative risk is only 12.0% for BC, and 1.3% for OC.[5] Furthermore, malignancy risk differs depending on various predictors (e.g., family history) and the location of mutations within the *BRCA1* and *BRCA2* genes.[4] It should be emphasised that deleterious *BRCA1* or *BRCA2* mutations can also augment the risk of breast and prostate cancer in men,[6] as well as pancreatic and stomach cancers,[7,8] or colorectal cancer, in both women and men.[9]

Poly(ADP-ribose) polymerases (PARP) are a family of enzymes that transfer adenosine diphosphate (ADP) ribose parts to surrounding proteins in response to different cellular stimuli. PARP1 and PARP2 participate in the cellular response to single-strand DNA breaks.[10] Inhibition of the ability of PARP to repair single-strand DNA breaks results in double-strand breaks (DSB) and subsequent replication fork collapse, which can lead to cell death. DSB can be repaired via different mechanisms, including the predominate means homologous recombination (HR) repair, which depends on *BRCA1* and *BRCA2* functionality.[10] Recent evidence has shown that PARP inhibitors (e.g., olaparib and talazoparib) are effective in treatment of malignancies that harbour deleterious g*BRCA*m compared to *BRCA* wild-type tumours (e.g., metastatic BC).[11,12]

The objective of this mini-review is to present the mechanisms of action of PARP inhibitors and briefly summarise the clinical trials leading to the approval of olaparib, talazoparib, rucaparib, and niraparib for treatment of *BRCA*-mutated, human epidermal growth factor receptor 2 (HER2)-negative metastatic BC. It should be noted that PARP inhibitors (which are well tolerated oral agents) are providing some reasonable hope for better outcomes and quality of life in a vulnerable population of patients with metastatic BC. In addition, this article discusses the efficacy, safety, and resistance to PARP inhibitors, focussing on women with metastatic BC.

THE ROLE OF POLY(ADP-RIBOSE) POLYMERASE ENZYMES IN DNA REPAIR AND THE INSIGHTS INTO TARGETED SYNTHETIC LETHALITY OF THE TUMOUR

To ensure genomic integrity, cells can apply different mechanisms that identify and repair DNA injuries caused by exogenous or endogenous factors via highly coordinated DNA damage response systems.[13] Several 'sensors' of DNA lesions are located in the cell nucleus, which communicate with effectors at the different cell sites.[14] In particular, PARP1 (a member of the superfamily of ADP-ribosyl transferases that transfer poly[ADP-ribose] [PAR] or mono-ADP-ribose) is one such nuclear protein that is activated by DNA breaks.[15] PARP1 is able to synthesise PAR chains, which are signals for the mobilisation of DNA repair proteins.[16] Similarly, PARP2 and PARP3 display DNA-dependent (ADP-ribose) transferase activity.[16] Poly-ADP-ribosylation (PARylation) relates to the covalent binding of negatively charged PAR on target proteins.[16,17] It should be underscored that PARylation may destabilise or stabilise protein-DNA interactions, activate target proteins, and induce protein degradation by the proteasome.[17] In addition, PARP proteins can regulate various cellular functions (e.g., DNA transcription and DNA damage response) via PARylation.[15-17]

At different points of the cell cycle, cells can apply several mechanisms for DNA repair. For instance, if single-stranded break repair is blocked and that cell tries to divide, the break can become double-stranded. At this point, if the cell has HR deficiency (HRD) it cannot survive. Moreover, cells that do not have BRCA1 or BRCA2 proteins (resultant of deleterious *BRCA* mutations) are very sensitive to PARP inhibition because they are unable to repair the DSB. This leads to synthetic lethality and cellular apoptosis.[10,13] The concept of synthetic lethality has initiated the development of an innovative, genomically targeted therapy for patients with cancers that harbour g*BRCA*m (e.g., metastatic BC and OC).[11,14] This therapeutic strategy is possible because the genomic instability of some cancer cells allows a novel class of medications, PARP inhibitors, to act specifically on tumour cells and spare healthy cells. In fact, many tumour cells with HRD are more susceptible to PARP inhibitors because of the fact that if DNA DSB are formed, and HR is the predominant repair mechanism in those cells, the unrepaired breaks are lethal. As a consequence, the patients with tumours harbouring deleterious *BRCA* mutations (e.g., germline or somatic, resulting in defective DSB repair by HR) can achieve the greatest clinical benefits.[13,16]

POLY(ADP-RIBOSE) POLYMERASE INHIBITORS: AN OVERVIEW OF THE MAIN CLINICAL TRIALS LEADING TO THEIR APPROVAL IN THE METASTATIC BREAST CANCER SETTING

Recently, the following PARP inhibitors have received approval by the U.S. Food and Drug Administration (FDA) and European Medicines Agency (EMA): olaparib, talazoparib, rucaparib, and niraparib (Table 1).[11,12,18-24]

Table 1: Poly(ADP-ribose) polymerase inhibitors for the treatment of patients with advanced breast and ovarian cancer (based on the main clinical trials leading to their approval).

PARP inhibitor	Clinical trial	*BRCA* mutation	1. FDA approval [month/year] - Treatment indications 2. EMA approval [month/year] - Treatment indications	Reference
Olaparib	OlympiAD, Phase III NCT02000622 Study 42, Phase II NCT01078662 Study 19, Phase II NCT00753545	Deleterious germline or somatic *BRCA* mutations.	1.FDA [January 2018] - g*BRCA*m, HER2-negative metastatic BC (previously treated with CHT) in the neoadjuvant, adjuvant, or metastatic setting. FDA [December 2014] - g*BRCA*m, advanced OC (treated with ≥3 prior lines of CHT). 2.EMA [October 2014] - maintenance treatment of relapsed, platinum-sensitive high-grade OC with mutations (germline or somatic) in *BRCA* genes.	11 20 19

Table 1 continued.

Olaparib	SOLO2, Phase III NCT01874353 Study 19, Phase II NCT00753545	No requirement for deleterious germline or somatic *BRCA* mutations.	1.FDA [August 2017] - maintenance treatment of recurrent OC (in a complete or partial response to platinum-based CHT). 2.EMA [May 2018] - maintenance treatment of platinum-sensitive relapsed high-grade OC (in complete or partial response to platinum-based CHT), regardless of *BRCA* status.	18 19
Talazoparib	EMBRACA, Phase III NCT01945775	Deleterious germline or somatic *BRCA* mutations.	1.FDA [October 2018] - g*BRCA*m, HER2-negative locally advanced or metastatic BC.	12
Rucaparib	ARIEL3, Phase III NCT01968213	No requirement for deleterious germline or somatic *BRCA* mutations.	1.FDA [April 2018] - maintenance treatment of recurrent OC (in a complete or partial response to platinum-based CHT).	21
Rucaparib	ARIEL2, Phase II NCT01891344 Study 10, Phase I/II NCT01482715	Deleterious germline or somatic *BRCA* mutations.	2.EMA [May 2018] - platinum-sensitive, relapsed or progressive, gBRCAm, high-grade OC (previously treated with ≥2 lines of platinum-based CHT, and unable to tolerate further platinum-based CHT). 1.FDA [December 2016] - g*BRCA*m OC (previously treated with ≥2 CHT lines).	22 23

Table 1 continued.

Niraparib	NOVA study, Phase III NCT01847274	No requirement for deleterious germline or somatic *BRCA* mutations.	1.FDA [March 2017] - maintenance treatment of recurrent OC (in a complete/ partial response to platinum-based CHT). 2.EMA [November 2017] - maintenance treatment of platinum-sensitive relapsed high-grade OC, in response (complete/partial) to platinum-based CHT.	24

BC: breast cancer; CHT: chemotherapy; EMA: European Medicines Agency; FDA: U.S. Food and Drug Administration; g*BRCA*m: germline *BRCA*-mutation; HER2: human epidermal growth factor receptor 2; HR: hormone receptor; OC: ovarian cancer (i.e., serous epithelial ovarian, fallopian tube, or primary peritoneal cancer); PARP: poly (ADP-ribose) polymerase.

Olaparib was the first PARP inhibitor approved for the treatment of BC, based on the OlympiAD, Phase III randomised controlled trial, which has demonstrated that PARP inhibition was superior in the metastatic setting (e.g., third-line therapy), in terms of efficacy and safety, to chemotherapy (CHT).[11] The CHT choices in OlympiAD included capecitabine, eribulin, and vinorelbine.[11] As a consequence, olaparib has been indicated for deleterious or suspected deleterious g*BRCA*m, in women with HER2-negative metastatic BC, who have been treated with CHT (e.g., in the neoadjuvant, adjuvant, or metastatic setting).[11] In addition, olaparib has been approved as maintenance therapy in patients with platinum-sensitive high-grade OC (with or without BRCA mutations) (Table 1).[18,19] Likewise, talazoparib has been approved for the treatment of women with deleterious g*BRCA*m, HER2-negative locally advanced, or metastatic BC (based on the EMBRACA, Phase III randomised control trial).[12] From a clinical point of view, the findings of the OlympiAD and EMBRACA trials appear encouraging; however, it should be pointed out that the efficacy of PARP inhibitors was not compared to that of platinum CHT, and thus, the OlympiAD and EMBRACA studies could not evaluate the relative benefits of PARP inhibitors and platinum-based CHT in BC patients with gBRCAm.[11,12]

ASSOCIATIONS BETWEEN GERMLINE *BRCA* MUTATIONS AND TRIPLE-NEGATIVE BREAST CANCER

BRCA1 and *BRCA2* play an important role in repairing DNA injuries.[25] It should be highlighted that the *BRCA1* mutations are strongly associated with triple-negative breast cancer (TNBC) (oestrogen receptor-negative, progesterone receptor-negative, and HER2-negative BC).[26] Conversely, the *BRCA2* mutations are mostly associated with hormone receptor-positive/HER2-negative BC.[26] TNBC is the predominant subtype in women with a g*BRCA*m.[26,27] However, the majority of basal-like BC are not in the *BRCA1* carriers group, and 20% of genomic instability in TNBC can be explained by *BRCA1* or *BRCA2* inactivation.[28] For instance, 5–10% of BC, and 80% of *BRCA1*-related BC, are TNBC.[26,27] It should be noted that in TNBC, the *BRCA*ness phenotype can be related to *BRCA* mutations, *BRCA1* promoter methylation, or low BRCA1 mRNA or protein expression.[27] Most *BRCA1* carriers have basal-like BC. Among patients

with TNBC, 14.6% have germline DNA repair gene mutations (e.g., 8.5% in *BRCA1*, 2.7% in *BRCA2*, and 3.7% in the *PALB2* [partner and localiser of *BRCA2*], BARD1 [*BRCA1* associated RING domain 1], *RAD51* [RAD51 recombinase], or *BRIP1* [(*BRCA1* interacting protein C-terminal helicase 1]).[25,28]

In a recent clinical trial, conducted among women with metastatic TNBC (with abnormal changes in DNA repair that were similar to those of *BRCA*-mutated tumours), 50% of the patients were treated with carboplatin, and the other 50% with docetaxel.[29] It was shown in this unselected study population, that carboplatin and docetaxel revealed similar efficacy. It should be underscored that in women with g*BRCA*m, carboplatin doubled the response rate compared to those from the docetaxel group (68% versus 33%). However, this clinical advantage was not reported in patients with *BRCA1* mRNA-low tumours, *BRCA1* methylation, or HRD.[29] It should be noted that with regard to olaparib, the reported response rate of 68% and the median progression-free survival (PFS) of 6.8 months were similar to those observed with carboplatin in metastatic TNBC.[11] Since platinum sensitivity is associated with tumour susceptibility to PARP inhibitors, hopefully future large scale trials will compare the platinum-based CHT with the PARP inhibitors and show the application of carboplatin in TNBC.

RESISTANCE TO POLY(ADP-RIBOSE) POLYMERASE INHIBITORS: CHALLENGES AND CONSIDERATIONS

PARP inhibitor resistance can develop via multiple mechanisms because BRCA1/2-deficient tumour cells can restore HR repair and stabilise their replication forks.[30] In addition, it should be noted that there is some cross-resistance between platinum-based CHT and PARP inhibitors; however, this cross-resistance is incomplete, and it is still unclear to what degree the platinum compounds and the PARP inhibitors 'operate' on the same pathway. For instance, a study in women with OC has shown that patients who have platinum-sensitive cancers have also higher overall response rates to single-agent PARP inhibition. Conversely, patients with platinum-resistant or refractory tumours have revealed lower response rates.[31] Unfortunately, not all patients with *BRCA* mutations are responsive to PARP inhibitors, indicating a potential role of the primary resistance to such a therapy. This may be attributable to the possibility that certain changes in the *BRCA* genes could have different functional influences on the individual response to PARP inhibitors. Moreover, an analysis of tumour biopsies has revealed some molecular mechanisms that can be 'in charge' of the primary and acquired resistance to PARP inhibitors. The most common acquired resistance mechanisms to PARP inhibitors consist of secondary mutations restoring the BRCA1 or BRCA2 protein functionality. Such mechanisms have been studied in patients with OC and BC (i.e., metastatic TNBC), who were resistant to platinum compounds.[32,33]

In a recent study, secondary somatic mutations that restored the open reading frame of *BRCA* or HR-related genes (e.g., *RAD51C* and *RAD51D*) were identified in patients with OC, who progressed during treatment with rucaparib.[34] Furthermore, in patients with *BRCA* somatic heterozygous disruption, tumour progression was related to a recovery of *BRCA* activity. In contrast, patients with a single-copy loss of chromosome 17 and a somatic nonsense *BRCA1* mutation (in the remaining allele) had a long-term response (>7 years) to olaparib. In this case, deletion of the wild-type allele resulted in the restoration to a functional gene.[35] Some other clinically relevant resistance mechanisms can involve mutations or downregulation of PARP enzymes.[36] It should be highlighted that HR gene sequencing, HR deficiency, genomic loss of heterozygosity tests, or some genetics scoring systems can allow detection of several patients as possible candidates for therapy with PARP inhibitors. However, such testing may still overlook some patients for whom the treatment with PARP inhibitors might be beneficial. Therefore, in the future, clinical studies should integrate all the data derived from DNA sequence and gene copy number variation that are relevant to other DNA repair mechanisms, such as nonhomologous end joining, alternative-nonhomologous end joining, and DNA damage regulatory gene processes.[34] In the meantime, g*BRCA*m can be considered

as an indicator for targeted therapy with PARP inhibitors.

POLY(ADP-RIBOSE) POLYMERASE INHIBITORS: CLINICAL IMPLICATIONS, CONCERNS, AND FUTURE DIRECTIONS

PARP inhibitors are generally well tolerated, and their adverse effects (AE) are mostly related to haematologic and digestive tract toxicity (Table 2).[37,38] Such AE can be successfully managed by adjusting doses, and using symptomatic medications or transfusions, if necessary.[37,38] Although PARP inhibitors have revealed therapeutic efficacy mostly in women with advanced BC who harbour g*BRCA*m, their effectiveness outside of g*BRCA*m carriers (e.g., in case of somatic *BRCA* mutations or other genetic mutations) is an important area of future studies. Furthermore, novel predictive biomarkers of responsiveness (beyond g*BRCA*m) are needed in patients with metastatic BC. In addition, exploring the use of PARP inhibitors in combination with other anticancer therapies presents an ongoing challenge. In practice, the PARP inhibitors can be used as single agents, according to the concept of synthetic lethality (because of the defects in HR).[39] Moreover, the PARP inhibitors can be used in combination with other treatments (e.g., CHT or radiotherapy) because PARP inhibitors augment DNA damage and contribute to an increase in overall DNA damage in tumour cells, even without the presence of HRD.[39]

Olaparib, applied as a single agent, has been beneficial in patients with advanced BC and OC, and the median PFS in the case of BC is approximately 6 months.[33] Similarly, talazoparib, which is the most potent PARP inhibitor, has been used as a single agent in women with BC harbouring g*BRCA*m, in which the median PFS was almost 9 months.[40] It should be underscored that for patients with advanced and metastatic BC, *BRCA* testing is very important, together with possible testing for other germline mutations since PARP inhibition is an effective targeted therapy for g*BRCA*m. However, some important questions, which will hopefully be answered in the future trials, involve the following issues:

- Which therapies are most optimal for combination with PARP inhibitors?
- Should PARP inhibitors be introduced into the early-stage BC treatment?
- How effective are PARP inhibitors in BC patients with prior exposure to platinum-based CHT?
- Can intermittent therapeutic dosing of PARP inhibitors be used?

CONCLUSION

For many patients with metastatic BC, treatment with PARP inhibitors can be more effective and less toxic than that of CHT. Because of the underlying defects in DNA repair, cancer cells with *BRCA* mutations are vulnerable to therapies that target PARP. In particular, for malignant tumours with HRD, PARP inhibitors induce synthetic lethality.

Some subtypes of BC with g*BRCA*m or functional (nonmutational) defects in BRCA proteins represent therapeutic targets for PARP inhibitors. Clinical benefits of PARP inhibitors have mostly been accomplished in *BRCA*-associated cancers (such as advanced BC and OC). BC with defects in a specific DNA damage repair pathway is particularly sensitive to targeted therapy with PARP inhibitors. For instance, olaparib (the first PARP inhibitor approved by the FDA) has currently been indicated for the treatment of patients with HER2-negative metastatic BC previously treated with CHT and who harbour deleterious g*BRCA*m. The recent OlympiAD trial has revealed a significant PFS benefit of olaparib compared with the CHT. In addition, PARP inhibitors may offer an effective and safe therapeutic option for women with TNBC. The most common AE of PARP inhibitors include anaemia, neutropenia, nausea, and vomiting.

It should be highlighted that identifying possible predictors of response to PARP inhibitors and strategies to overcome various mechanisms of resistance merit investigation in the future clinical trials. In particular, the HRD assays attempt to use chromosomal instability as a marker. Also, there are many candidate genes beyond *BRCA1/2* that are involved in HR (e.g., *RAD51D*). Advances in genome-sequencing of tumour DNA, combined with modern

bioinformatics, will hopefully contribute to defining a more precise panel of genes that will be helpful in determining profiles of individual patients who may favourably respond to the therapy with PARP inhibitors.

Table 2: Poly(ADP-ribose) polymerase inhibitors: Dosing schedules, adverse effects, and special precautions.

PARP inhibitor	Olaparib (Lynparza)[11,37,38]	Talazoparib (Talzenna)[12]	Rucaparib (Rubraca)[21-23]	Niraparib (Zejula)[24]
Dosing schedule	300 mg PO bid	1 mg PO qd	600 mg PO bid	300 mg PO bid or qd
Adverse effects	Anaemia, leukopenia, fatigue, nausea, vomiting, diarrhoea, abdominal pain.	Fatigue, nausea, headache, alopecia, anaemia, neutropenia, thrombocytopenia, vomiting, diarrhoea, decreased appetite.	Anaemia, leukopenia, fatigue, increased sensitivity to sunburn, nausea, vomiting, diarrhoea, abdominal pain.	Nausea, thrombocytopenia, fatigue, anaemia, constipation, vomiting, abdominal pain, neutropenia.
Special precautions	**Pneumonitis** - interrupt treatment if pneumonitis is suspected, discontinue if it is confirmed; **MDS/AML (rare)** - if confirmed, discontinue olaparib; combination of olaparib with other DNA damaging agents can increase **myelosuppressive toxicity;** coadministration of CYP3A4 inhibitors can increase olaparib plasma concentrations (CYP3A inhibitors should be avoided, or the dose of olaparib has to be reduced, e.g., to 100 mg PO bid).	Coadministration with amiodarone, carvedilol, verapamil, clarithromycin, or itraconazole should be avoided; however, if these agents have to be used, the dose of talazoparib should be reduced (e.g., to 0.75 mg qd).	**MDS/AML (rare)** - if confirmed, discontinue rucaparib; advise patients to use sun protection.	**MDS/AML (rare)** - if it is confirmed, discontinue niraparib; **Hypertension and hypertensive crisis** - use antihypertensive medications and adjust dose of niraparib; **Haematologic adverse reactions** (e.g., if platelet count is ≤10,000/mcL - consider platelet transfusion, or if other agents, such as anticoagulant or antiplatelet agents are used, interrupt anticoagulants or antiplatelet agents or transfuse if necessary).
Monitoring tests	CBC count for cytopenia - at baseline and then monthly; patients have to recover from haematological toxicity (e.g., because of previous therapy) before starting a PARP inhibitor.	CBC count for cytopenia at baseline and then monthly; patients have to recover from haematological toxicity (e.g., because of previous therapy) before starting a PARP inhibitor.	CBC count for cytopenia at baseline and then monthly; patients have to recover from haematological toxicity (e.g., because of previous therapy) before starting a PARP inhibitor.	Monitor BP and pulse monthly for the first year and periodically thereafter during treatment.

AML: acute myeloid leukaemia; bid: twice a day; BP: blood pressure; CBC: complete blood count; CHT: chemotherapy; ET: endocrine therapy; HER2: human epidermal growth factor receptor 2; HR: hormone receptor; MDS: myelodysplastic syndrome; PARP: poly (ADP-ribose) polymerase; PO: per os (orally); qd: once a day.

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Branchial Cleft Cyst – A Misdiagnosed Developmental Anomaly

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Disclosure: The authors have declared no conflicts of interest.

Received: 09.04.19

Accepted: 23.08.19

Keywords: Branchial cleft cyst, congenital cyst, surgical excision.

Citation: EMJ Oncol. 2019;7[1]:77-81.

Abstract

Branchial cysts appear most often as unilateral neck masses and account for 25% of head and neck congenital swellings, of which 95% arise from the second branchial cleft. Here, the authors report a rare case of branchial cleft cyst in a 16-year-old girl, which is often misdiagnosed and treated improperly.

INTRODUCTION

The branchial cleft's embryological journey begins between the first 3–8 weeks of intrauterine life. Five mesodermal arches form by the inpouching of ectodermal clefts and endodermal pouches; however, incomplete obliteration of these clefts result in anomalies such as cervical lymphoepithelial cysts, branchial cysts, sinus tracts, or fistulae.[1,2] The occurrence of branchial cleft cysts shows no significant gender predilection and presents in young adults with peak incidence during the third decade of life.[2-4] Branchial cysts appear most often as unilateral neck masses and account for 25% of head and neck congenital swellings, of which 95% arise from the second branchial cleft.[4]

CASE REPORT

A 16-year-old girl reported to the hospital with a swelling in the right side of the neck which was present from childhood and was slowly increasing in size. Her medical history was insignificant. On clinical examination there was 5x4 cm round swelling in the right side of the neck below the sternomastoid muscle, and was non-tender, non-pulsatile, fluctuant, and soft in consistency (Figure 1). There were no associated complaints such as pain, change in voice, or difficulty in breathing. The swelling was not moving with protrusion of tongue or on deglutition, and there was no history of discharge during eating or drinking. A clinical diagnosis of congenital cyst or paraganglioma was made. Pre-operative haematological investigations were carried out and were within normal limits. Ultrasonography (US) guided fine needle aspiration cytology (FNAC) revealed a milky, white-coloured fluid which was confirmed as a branchial cleft cyst. A contrast MRI was performed to find the extent of the cyst and its relation to the internal jugular vein and carotid artery (Figure 2). Surgical removal was planned, and a submandibular incision was directed to the

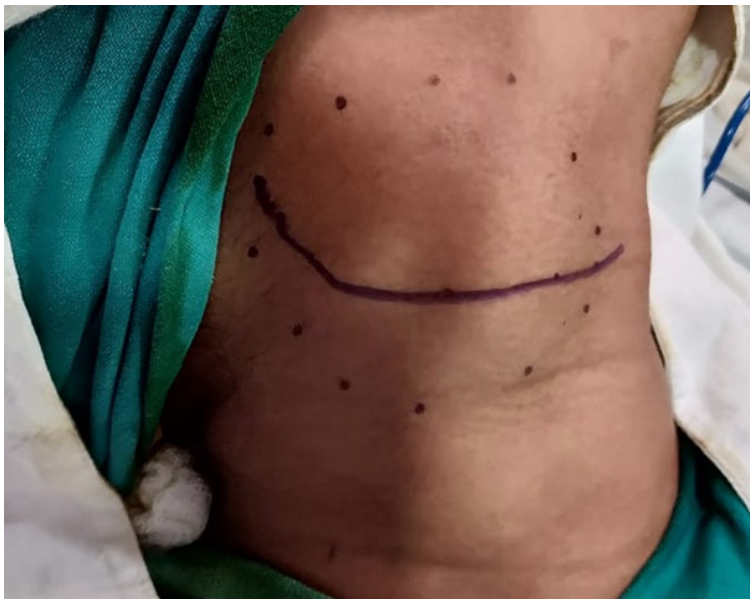

Figure 1: 5x4 cm round swelling in right side of the neck below the sternomastoid muscle, non-tender, fluctuant, and soft in consistency.

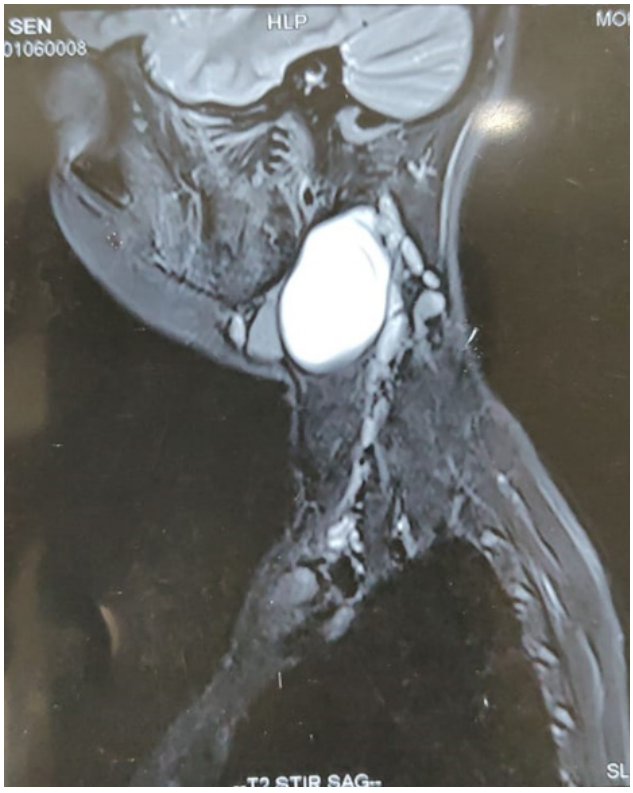

Figure 2: MRI with contrast was carried out to find the extent of the cyst and its relation to the internal jugular vein and carotid artery.

right side of the neck. Dissection was performed carefully to avoid puncture of the cyst. The cyst was detached from the base and enucleation was completed (Figure 3). All the major vessels and cranial nerves were saved. The final histopathological report confirmed the swelling as a branchial cleft cyst. Drain number 12 was kept and closure was completed in layers.

DISCUSSION

The earliest report of a branchial cyst was by Hunczovsky in 1785.[1] These cysts are common but often present diagnostic dilemmas to clinicians and radiologists and challenge surgeons because of the close anatomical presence to vital neurovascular elements. It has become imperative to document each variation and occurrence of these cysts closely to develop a protocol and strategy for effective management.[5-7]

Understanding of the pathophysiology of these cysts is paramount to understanding them as an entity. Various theories have been proposed to explain the origin of branchial cysts, including their consideration as epithelial inclusions within a lymph node, or alternatively as remnants of an original connection between the thymus and third branchial pouch. Other theories include the cervical sinus theory and the branchial apparatus theory. The cervical sinus theory suggests the origin of these cysts is from the remains of the cervical sinus of His, formed by the growing down of the second arch and its fusion with the fifth arch. Conversely, the branchial apparatus theory states that the origin is routed in pharyngeal pouches or branchial clefts.[8-10] This case is rare in the authors' surgical setting and here the detailed sequence of its management is reported.

MANAGEMENT OF BRANCHIAL CLEFT CYST

Evaluating a patient suspected with having a branchial cleft will involve a conglomeration of clinical examination and imaging modalities. The clinical and most commonly diagnostic signs include a slow progressing mass that can be present from weeks to years with overlying normal skin. Palpation reveals smooth, round, soft, non-tender, fluctuant, mobile, and asymptomatic masses. These cysts rarely have potential for malignant transformation and rupture. Primary branchial cleft cyst is typically located between the external auditory canal and submandibular area and it is usually in close proximity to the parotid gland and facial nerve.[10,11] It has two types of presentation; Type 1 is characterised by duplication of the membranous external auditory canal; and Type 2 is composed of ectomesoderm and cartilage. The clinical presentation is usually soft tissue mass or draining sinus in the retromandibular region accompanied by ear discharge. Secondary brachial cleft cysts lie between the lower anterior border of the sternocleidomastoid and the tonsillar fossa, close to the glossopharyngeal and hypoglossal nerve, and carotid vessels. They become tender if secondarily inflamed or infected. Inspect for mucoid or purulent discharge on the skin or into the pharynx via draining sinus tracts.[12]

The authors relied on initial fine needle aspiration to distinguish a cleft cyst from malignancy when carrying out radiographic assessment. Contrast enhanced CT, US, and, MRI also significantly contributed to arriving at a preoperative diagnosis and treatment trajectory. Odontogenic infection, cystic hygroma, enlarged lymph nodes, paragangliomas of the vagus nerve, lipoma, carotid body tumours, neurofibroma, haemangioma, lymphangioma, teratoma, ectopic salivary tissue, pharyngeal diverticulum, laryngocele, saccule, and cystic schwannoma[13] should be ruled out as they constitute the differential diagnostic possibilities of a branchial cleft cyst.[14] Elective excision is the most favourable treatment for a branchial cleft cyst because of the risk of infection, further enlargement, or malignancy. Aesthetic concerns entail the use of a transverse incision directly over the cyst. Care should be taken to avoid important vascular structures such as the internal and external carotid arteries, and nerves like the superior laryngeal, glossopharyngeal, vagus, and hypoglossal nerve. Surgical complications arise occasionally, and one must be prepared to manage events like recurrence, persistent fistula, and damage to the cranial nerves.[12]

CONCLUSION

Effective management of branchial cleft cysts predominantly rests on surgical precision which improves with proper understanding

of the entity coupled with good radiographic assistance. As branchial cleft cyst has many differential diagnoses, it is important to confirm the diagnosis by histopathological examination of the excised tissue.[13]

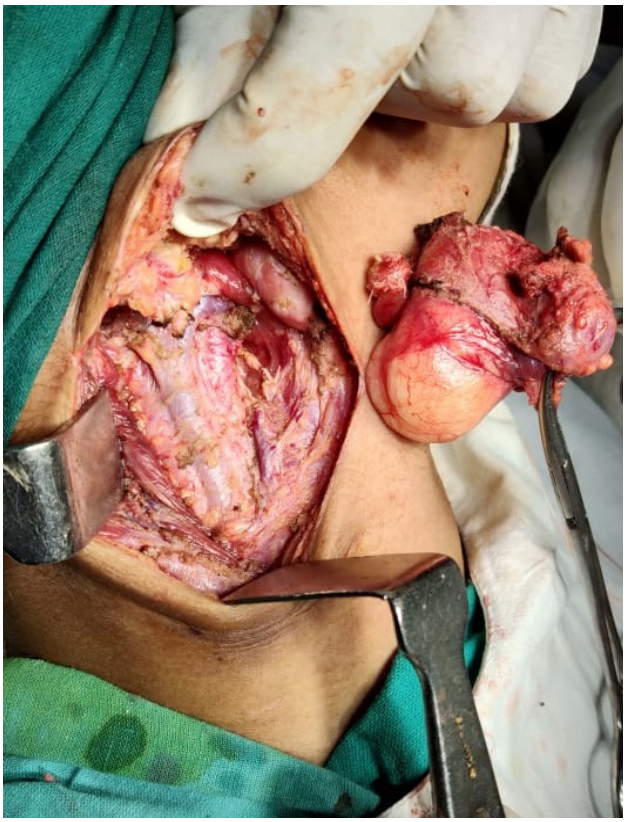

Figure 3: The cyst was detached from the base and enucleation was complete. All vital neuro-vascular structures were saved.

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Discovery of a 'Grail-Shaped' Drug: Ne-ratinib and the Downregulation of Mutant RAS

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Disclosure: The authors have declared no conflicts of interest.

Acknowledgements: Support for the present study was funded from philanthropic funding from Massey Cancer Center and the Universal Inc. Chair in Signal Transduction Research. Dr Dent acknowledges funding by the Commonwealth Health Research Board (CHRB) of Virginia.

Received: 28.06.2019

Accepted: 12.09.2019

Keywords: Hippo, histone deacetylase (HDAC) inhibitor, neratinib, RAS, sildenafil, yes-associated protein (YAP), statin.

Citation: EMJ Oncol. 2019;7[1]:81-89.

Abstract

It has been stated that developing a drug that can attack mutated RAS proteins is 'the Holy Grail' of cancer therapeutics. Through a series of unexpected findings, the authors discovered that the irreversible epidermal growth factor receptor 1/2/4 inhibitor neratinib (HKI-272, Nerlynx®) was not only an inhibitor of those receptor tyrosine kinases, but could additionally cause receptor internalisation and degradation. To the author's surprise, the negative control receptors c-MET and c-KIT were also degraded after neratinib exposure, albeit with a slower time-course. This appeared to be attributable to neratinib attacking receptor tyrosine kinases localised in quaternary structures. It was reasoned that neratinib had the potential to downregulate the expression of other plasma membrane localised signalling proteins, particularly RAS. In a variety of tumour types, neratinib could reduce the expression of wild type (Kirsten) and mutant (Neuroblastoma) RAS (K-RAS/N-RAS, respectively). It was subsequently demonstrated that mutant Gα proteins in uveal melanoma could also have their expression reduced by neratinib. Neratinib was shown to be an inhibitor of sterile 20 serine/threonine kinases. Acting as an inhibitor of sterile 20 serine/threonine kinases, combined with RAS inhibition, neratinib enhanced the phosphorylation and degradation of the Hippo pathway effectors yes-associated protein and transcriptional coactivator with PDZ-binding motif. In malignancies expressing a mutant K-RAS, yes-associated protein and transcriptional coactivator with PDZ-binding motif are localised in the nucleus where they cooperate with mutant K-RAS signalling to promote growth, invasion, and chemotherapy resistance. Thus, whilst neratinib is not a direct inhibitor of mutant RAS signalling, the Holy Grail, it nonetheless represents, as did the beacon atop Castle Anthrax, at least something 'Grail-shaped'.

WE ARE THE SCIENTISTS WHO SAY NERATINIB

The drug neratinib (HKI-272, Nerlynx®) was originally developed by Wyeth Research Laboratories, with its initial characterisation published in 2004.[1] Several years later, as part of a comprehensive screening study examining the inhibitory properties of 40 kinase inhibitors against approximately 400 kinases, neratinib was shown, using computational chemical biology techniques, to be a potent inhibitor. This was not only of the receptor tyrosine kinases epidermal growth factor receptor 1/2/4 (ERBB1/2/4), but also of multiple sterile 20 (Ste20) serine/threonine kinases.[2] In the case of MAP4K5, neratinib was claimed to be a more potent inhibitor of this kinase compared to ERBB1. In the case of mammalian Ste20-like protein kinase 3 (MST3) and MST4, neratinib had a similar efficacy for their inhibition as the drug did for ERBB2 (Figure 1). Despite this surprising observation, no published studies have yet explored this possible 'off-target' biology surrounding the actions of neratinib. Subsequently, neratinib received U.S. Food and Drug Administration (FDA) approval as a neoadjuvant therapy in HER2+ breast cancer.[3]

The authors' initial interest in neratinib was based upon data demonstrating that ERBB1/2/4 inhibitors, particularly the irreversible inhibitor afatinib, could combine with the JAK1/2 inhibitor ruxolitinib to kill tumour cells.[4] Afatinib is approved as a non-small cell lung cancer (NSCLC) therapeutic, and in another project, multiple independent afatinib-resistant NSCLC clones had been generated from *in vivo* exposure of established H1975 tumours that express ERBB1 L858R/T790M.[5] Characterisation of these cells revealed no additional adaptive mutations in cancer hot-spot genes, and instead, these cells had reduced their expression of the tumour suppressor phosphatase and tensin homolog (PTEN) and increased expression of the PTEN-regulatory E3 ligase NEDD4.[6] Neratinib and afatinib were both capable of killing parental H1975 clones, but only neratinib killed the afatinib-resistant H1975 clones; additionally, the resistant cells were significantly more sensitive to neratinib even though they expressed less of the proposed primary neratinib targets ERBB1/2/4. These findings implied that neratinib "had to be inhibiting something else" so that it could kill the afatinib-resistant NSCLC cells.

RAS: THE ONCOGENE SUPREME

Very few modern targeted cancer therapeutics have significant clinical activity when used as a stand-alone medication. In general, where single agent drugs have shown activity, the tumour cells exhibited an exquisite addiction to signals emanating from one particular mutated enzyme, e.g., BCR-ABL; ERBB1 L858R.[7] One family of oncogenes that are rational single agent targets, but that have proven very difficult to block, are those belonging to the RAS family.[8-10] RAS proteins are small GTPases that regulate cellular signalling cascades downstream of receptor tyrosine kinases to control cell growth, proliferation, and differentiation.[11-14] The three RAS isoforms H (Harvey), N (Neuroblastoma), and K (Kirsten)-RAS are expressed in mammalian cells; for example, K-RAS is mutated in 90% of pancreatic tumours.[15] In mutated RAS proteins, the GTPase activity of the protein is greatly reduced, and thus the RAS protein is permanently capable of activating downstream effectors such as RAF-1.[16] To act as signal transducers, RAS proteins must also be localised to the inner leaflet of the plasma membrane by a COOH-terminal membrane lipid anchor. For K-RAS, the anchor comprises a covalently attached COOH-terminal cysteine farnesyl-methyl ester operating together with a polybasic motif of 6 lysine residues that provide electrostatic membrane affinity. Clinically relevant β-hydroxy β-methylglutaryl-CoA reductase inhibitors, i.e., statins, reduce the levels of farnesyl substrate and reduce the amount of K-RAS that can localise in the plasma membrane.[17]

Although for nearly 40 years oncogenic forms of RAS have been recognised as key drivers of cancer growth, invasion, and chemotherapy resistance, they have also been considered as 'undruggable'.[18-20] Hence, therapeutic inhibition of mutant RAS signaling came to be considered as the 'Holy Grail' in the field of cancer therapeutics.[21] In recent years, attempts

have been made to develop agents that directly inhibit mutant RAS function, but these inhibitors at present only target a small fraction of RAS mutations.[22-24] Initial studies with neratinib demonstrated that it not only blocked the kinase activities of ERBB family receptors, but that it also caused their degradation.[25] In agreement with the concept that receptors reside in quaternary structures in the plasma membrane, neratinib also causes the degradation of c-MET and c-KIT that do not bind the drug. Based on these findings, it was reasoned that other signal transducing proteins, i.e., RAS isoforms, may also become degraded after neratinib exposure. Clinically relevant concentrations of neratinib within 4 hours reduced the expression of mutant K-RAS proteins in pancreatic cancer cells by 30%; this down-regulation only resulted in 15–20% tumour cell death after 24 hours.

RAS: ADDITIONAL OPPORTUNITIES TO ATTACK ITS ONCOGENIC CAPABILITY

In the case of neratinib and mutant K-RAS, two additional concepts were then developed to enhance the downregulation effect and to increase the killing. The combination of neratinib with histone deacetylase (HDAC) inhibitors, including sodium valproate, entinostat, panobinostat, and vorinostat, resulted in a rapid 50–70% reduction in K-RAS expression associated with >40% of the cells dying within 24 hours.[26,27] The second concept involved directly attacking the mutant K-RAS protein itself through mechanisms independent of those induced by neratinib. K-RAS is phosphorylated by protein kinase G, a downstream target of phosphodiesterase 5 inhibitors such as sildenafil (Viagra®), which leads to K-RAS leaving the plasma membrane.[28] For K-RAS to be localised in the plasma membrane it must be prenylated, and inhibitors of β-hydroxy β-methylglutaryl-CoA reductase, including statins such as atorvastatin (Lipitor®), reduce farnesyl substrate levels required for the prenylation of K-RAS.[29] Neratinib, sildenafil, and atorvastatin interacted in a greater than additive fashion to reduce K-RAS protein levels and to kill pancreatic cancer cells (Figure 2).[27] One observation from preclinical *in vivo* studies in triple-negative breast cancer cells was that tumours previously exposed to neratinib and the HDAC inhibitor sodium valproate had permanently reduced their expression of ERBB1, K-RAS, and N-RAS 14 days after the final treatment.[30] Thus, this drug combination appeared to have evolved the surviving tumour cells *in vivo* to have a less aggressive phenotype. A Phase I trial recently opened at Massey Cancer Center combining neratinib with the HDAC inhibitor valproate,[31] with planned expansion cohorts at the recommended Phase II dose in patients carrying K-RAS-mutant tumours. One pancreatic adenocarcinoma patient, in the first cohort, has exhibited stable disease.

NOT THE COMFY CHAIR: NERATINIB IS A MULTI-KINASE INHIBITOR

As mentioned in the first paragraph, in addition to ERBB1/2/4, neratinib could potentially inhibit MAP4K serine/threonine kinases. These alternate/secondary neratinib MAP4K targets are not only expressed in epithelial carcinoma cells but also in haematopoietic tumour cells. This raised the possibility that neratinib, alone or combined with HDAC inhibitors, could be repurposed as an antileukemia or an antilymphoma drug. Not only did neratinib and HDAC inhibitors interact to kill acute promyelocytic leukaemia, acute myeloid leukaemia, and T cell lymphoma cells, the drugs interacted, in the absence of any ERBB family receptor expression, to reduce the protein levels of K-RAS and N-RAS in these cells. These findings suggested that specific inhibitors of MAP4K/Ste20 kinases, more potent and efficacious than neratinib, may have a wide utility as cancer therapeutics.

Canonical Hippo pathway signalling initially involves the MAP4K family enzymes MST1 and MST2 phosphorylating the intermediate kinases large tumour suppressor kinase (LATS) 1/2 and the docking protein/chaperone MOB1. LATS1/2 phosphorylate the yes-associated protein (YAP) and transcriptional coactivator with PDZ-binding motif (TAZ) effector co-transcription factors.[32-40] Phosphorylated YAP and TAZ are located in the cytosol whereas dephosphorylated YAP/TAZ are nuclear and regulate transcription. Phosphorylated YAP/TAZ in the cytoplasm are degraded via ubiquitination (Figure 1). In malignancies expressing a mutant K-RAS, YAP and TAZ

are predominantly localised in the nucleus where they cooperate with mutant K-RAS signalling to promote growth, invasion, and chemotherapy resistance.[38,39] High expression levels of YAP are clinically associated with greater metastatic spread of pancreatic cancer cells,[39] and downstream of mutant K-RAS, the transcription regulator YAP is essential for neoplastic progression to pancreatic ductal adenocarcinoma.[40,41] Well-described proteins whose expression is regulated by the Hippo pathway include cyclin E, cell-division cycle protein 20, solute carrier family 7 member 5, anillin, stathmin 1, SH2 domain-binding protein 1, N-myc downstream-regulated gene 1, collagen Type IV alpha-3, fascin, hyaluronan-mediated motility receptor, moesin, cytochrome P450, shisa family member 4, growth differentiation factor 15, and damage specific DNA binding protein 2.[41-47] Cell-division cycle protein 20 overexpression in pancreatic cancer predicts for poor patient survival. Overexpression of the L-amino acid transporter solute carrier family 7 member 5 predicts for poor pancreatic patient survival. Elevated levels of the cytoskeletal protein stathmin 1 is associated with poorer patient survival. Transforming growth factor beta family member growth differentiation factor 15 is overexpressed in pancreatic cancer and has been proposed as a better biomarker for pancreatic cancer than CA19-9. N-Myc downstream regulated 1 is a key mammalian target of rapamycin (mTOR) complex 2 effector that regulates the stability of methyltransferases and alkylating agent resistance.

Neratinib reduced the phosphorylation of MST1, MST3, and MST4, yet increased the phosphorylation of LATS1/2. LATS1/2 activation correlated with enhanced phosphorylation and degradation of YAP and TAZ, an effect that was further increased when it was combined with an HDAC inhibitor (Figure 1).[27] This implied that neratinib may cause a compensatory activation of an unknown MAP4K that can act to phosphorylate LATS1/2. The authors also determined whether neratinib, atorvastatin, and sildenafil could interact to alter YAP phosphorylation. Both atorvastatin and neratinib enhanced YAP phosphorylation, though neratinib more effectively suppressed K-RAS expression. The drugs interacted to further elevate YAP phosphorylation and to reduce K-RAS expression. Sildenafil enhanced the ability of neratinib plus atorvastatin to further suppress K-RAS expression and to increase YAP phosphorylation. Thus, the three agents in coordination acted to simultaneously block RAS and YAP function which was associated with significant amounts of tumour cell killing (Figure 2).

AUTOPHAGY: EVOLUTIONARY RECYCLING AND A CANCER CELL'S ACHILLES HEEL

Autophagy is an evolutionary conserved process found in single cell yeasts and in multicellular mammals.[48,49] The basic role of autophagy is to recycle cellular components if they are damaged or denatured, or during times of nutrient stress, to maintain homeostasis and cell viability.[50] The autophagosome initially forms around the damaged organelles and/or proteins, and the fuses with acidic endosomes to form an autolysosome.[51] The organelles and proteins are subsequently degraded in the autolysosome and the degraded materials returned to the cell for new uses.[52] The regulation of autophagy in mammalian cells can be simplistically viewed as alterations in signalling by mTOR that regulates the expression and phosphorylation of multiple proteins who control autophagosome formation, autophagosome fusion with endosomes, and autolysosome acidification.[53-57] Simplistically, mTOR and the AMP-dependent protein kinase (AMPK) coordinately regulate the activity of the Unc-51 like autophagy activating kinase (ULK1). Phosphorylation of ULK1 at COOH-terminal sites by mTOR inactivates ULK1, for example at S757. Phosphorylation of ULK1 at sites closer to the NH2-terminus of the protein by the AMPK activates ULK1, for example at S317. Furthermore, it should be noted that signalling by the AMPK can itself cause inactivation of mTOR via phosphorylation of raptor; mTOR activity is generally thought to be maintained by upstream signalling from the PI3K/PTEN/protein kinase B pathway. This dynamic multi-site phosphorylation of ULK1 means that a cell can exquisitely control the ability of ULK1 to phosphorylate its key target: autophagy-related protein 13. Phosphorylation of autophagy-related protein 13 at serine 318 represents the key gate-keeper step for autophagosome

formation. Important additional proteins, such as Beclin1 and autophagy related 5, also play essential roles in the formation of the double-membrane autophagosome. Under normal biological circumstances, the autophagosome matures and then fuses with endosomes that acidify, facilitating the proteolytic degradation of their contents, for example by cathepsin and calpain proteases.[58-61] This transition process is termed 'autophagic flux'.

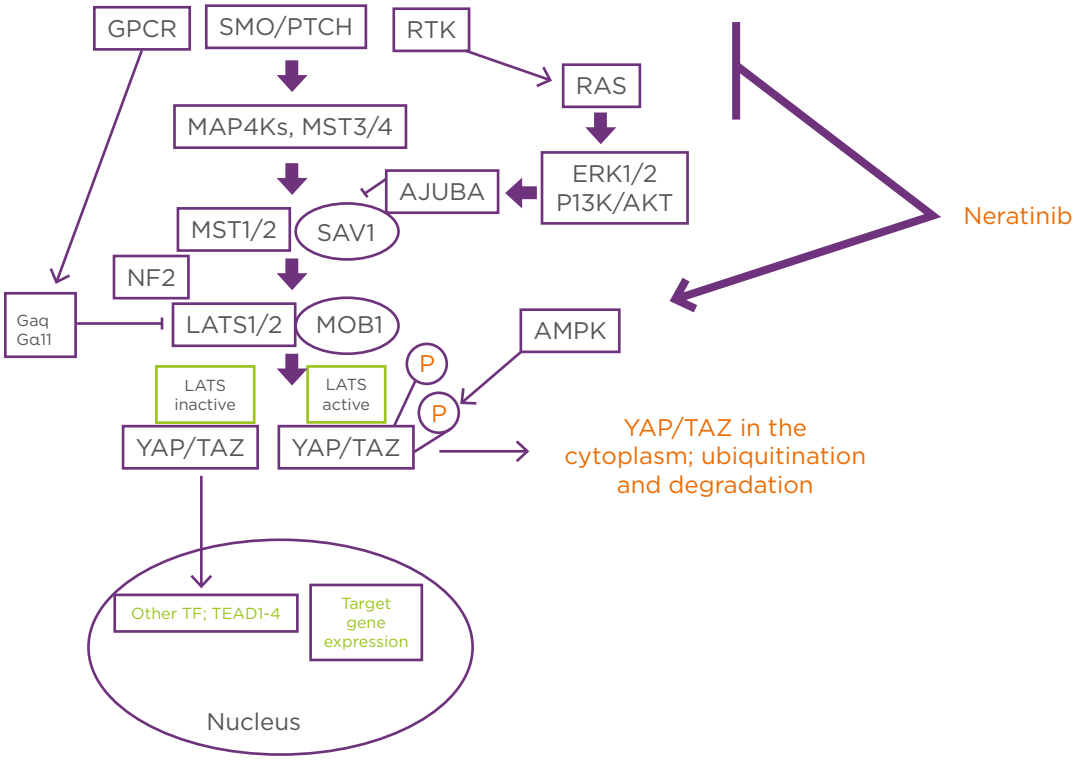

Figure 1: Putative mechanisms by which neratinib could coordinately control mutant RAS and Hippo Pathway signaling.

Signals from plasma membrane receptors can either stimulate or inhibit Hippo pathway functionality. Classic Hippo pathway signalling proceeds from Smoothened and Patched and via the regulation of MAP4K results in the phosphorylation and activation of MST1/2. Activated MST1/2 phosphorylate and activate LATS1/2. Activated LATS1/2 phosphorylate YAP and TAZ, which causes cytoplasmic sequestration of YAP and TAZ, prior to their eventual degradation. Through a variety of mechanisms, the activities of MST1/2 and LATS1/2 can be reduced via crosstalk from other signaling pathways. Thus, mutant RAS signaling, via ERK and AKT signaling and a redistribution of chaperoning/complex effectors, can block MST1/2 signaling. Mutant Gα proteins, as found in uveal melanoma, can act to prevent LATS1/2 activation. Reduced MST/LATS activities result in YAP/TAZ dephosphorylation, permitting these co-transcription factors to interact with TEAD proteins to enhance the expression of proteins that promote growth, invasion and chemotherapy resistance. Neratinib can block the actions of mutant RAS as well as of mutant Gα proteins. Furthermore, it activates the AMPK, all of which would be predicted to prevent YAP/TAZ and TEAD proteins colocalising in the nucleus where they facilitate tumourigenic cellular behaviour.

AKT: protein kinase B; AMPK: 5′ AMP-activated protein kinase; ERK: extracellular regulated kinase; GPCR: G-protein-coupled receptors; LATS: large tumour suppressor kinase; MOB: MOB kinase activator; MST: mammalian Ste20-like protein kinase; NF2: neurofibromatosis type 2; P: phosphorylated; PTCH: Patched; RTK: receptor tyrosine kinase; SAV1: salvador homolog 1; SMO: Smoothened; TAZ: transcriptional coactivator with PDZ-binding motif; TEAD: TEA domain family member 1; TF: transcription factor; YAP: yes-associated protein.

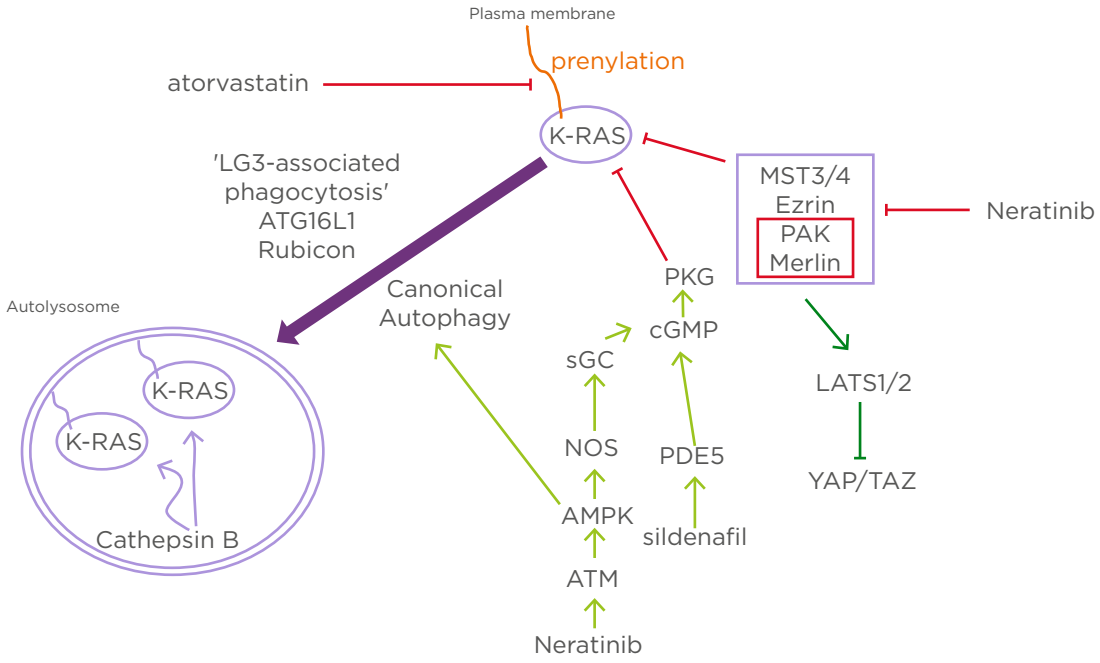

Figure 2: Putative mechanisms by which neratinib downregulates mutant Kirsten-RAS expression and inactivates Hippo pathway function.

Neratinib via inhibition of MST3/4 causes Rubicon-dependent phagocytosis and in parallel, neratinib activates ATM-AMPK signaling that causes autophagosome formation. Collectively, this results in the cathepsin-dependent degradation of mutant K-RAS. Atorvastatin via reduced prenylation and sildenafil via increased PKG-dependent phosphorylation of K-RAS also independently act to lower mutant K-RAS levels. As part of this process at the plasma membrane, neratinib reduces PAK1 phosphorylation that leads to dephosphorylation of the PAK1 substrate Merlin/NF2. Dephosphorylated Merlin facilitates activation of LATS1/2, and active LATS1/2 phosphorylate YAP and TAZ. Phosphorylated YAP and TAZ leave the nucleus, preventing them from acting as transcriptional coactivators.

AMPK: 5′ AMP-activated protein kinase; ATG16L1: autophagy related 16 like 1; ATM: Serine-protein kinase ATM; cGMP: cyclic guanosine monophosphate; K: Kirsten; LATS: large tumour suppressor kinase; LC3: microtubule-associated proteins 1A/1B light chain 3B; MST: mammalian Ste20-like protein kinase 3; NOS: nitric oxide synthases; PAK: Serine/threonine-protein kinase, PDE5: phosphodiesterase type 5 inhibitor; PKG: protein kinase G; sGC: soluble guanylate cyclase; TAZ: transcriptional coactivator with PDZ-binding motif; YAP: yes-associated protein.

Neratinib rapidly promoted the formation of autophagosomes, which was attributable to both activation of an ATM–AMPK–ULK1 pathway and by reduced protein kinase B and mTOR signaling.[25-27] This autophagy effect was significantly enhanced by combined exposure of neratinib with HDAC inhibitors. Because the authors were using HDAC inhibitors, control studies were performed to examine the total expression of the various HDAC proteins, 1–11, in the tumour cells. The authors discovered that the combination of neratinib with HDAC inhibitors caused the protein expression of multiple HDAC proteins to rapidly decline, particularly HDAC1/2/3/6, an effect that was blocked by preventing autophagosome formation. For example, the expression of cytosolic HDAC6 and the chaperone it regulates, HSP90, were rapidly reduced via this process. The loss of HDAC6/HSP90 function has been shown by many laboratories to be highly detrimental to tumour cell growth and the maintenance of drug-resistance.[62] As HDAC6/HSP90 function declines, the amount of unfolded protein within the cytoplasm and the endoplasmic reticulum increases, leading to prolonged intense PKR-like endoplasmic reticulum kinase-eIF2α endoplasmic reticulum stress signalling, and a dramatic reduction in the levels of protective proteins with short half-lives such as myeloid leukaemia cell differentiation protein-1.[25,63,64]

HDAC INHIBITORS AS ENHANCERS OF IMMUNOTHERAPY EFFICACY

HDAC inhibitors have generated interest within the checkpoint immunotherapy field where it has been argued that epigenetic modulation of protein expression by this family of drugs predisposes tumours to be more responsive to immunotherapeutic checkpoint inhibitory antibodies.[65-67] In prior studies from this laboratory, using drug combinations such as neratinib plus valproate but also with combinations that lack an HDAC inhibitor (e.g., pemetrexed plus sildenafil), it was observed that the drug combinations simultaneously rapidly enhanced, again in an autophagy-dependent fashion, tumour cell expression of Class I major-histocompatibility, and decreased expression of programmed death ligand 1, ornithine decarboxylase, and indoleamine 2, 3-dioxygenase 1.[25,63,66-68] Because the drug combination was reducing the levels of HDAC proteins via autophagy whilst simultaneously altering the expression of immunologically important proteins, the authors hypothesised that these two events were linked. Using molecular tools to knock down the levels of HDAC 1/2/3/10, alone or in combination, the authors recapitulated the effects on immunological protein expression that were observed following drug combination exposure. Thus, for drug combinations lacking any epigenetic modulator, to cause these changes in protein expression, required autophagosome formation; i.e., the drug-combinations reduced the levels of HDAC and HDAC expression was preserved when drug-induced autophagosome formation was blocked. These findings imply that any drug combination which causes prolonged endoplasmic reticulum stress signalling together with strongly enhancing autophagosome formation have the potential, via down-regulation of HDAC proteins themselves, to both cause tumour cell death in parallel with altering epigenetics/transcription and protein expression leading to enhanced tumour cell immuno-sensitivity.

For neratinib plus valproate, beyond showing that the two drugs interacted to suppress tumour growth, additional studies using checkpoint inhibitory immunotherapy antibodies were performed.[25] Under controlled antibody conditions, a 3-day exposure of tumours to neratinib plus valproate, 13 days later, still resulted in significant reductions in the expression of K-RAS and ERBB1. The expression of several HDAC proteins, such as HDAC6, was also permanently reduced. These findings suggested that at least for this particular drug combination the expression of some oncogenes can be 'reset' to basal levels. Prior exposure of tumours to neratinib plus valproate enhanced the antitumour efficacy of both an anti-programmed cell death protein 1 antibody and an anti-cytotoxic T-lymphocyte-associated protein 4 antibody. These events were associated with immune cell infiltration into the tumour: M1 macrophages, activated natural killer cells, and CD8 T cells, all of which correlate with the observed antitumour response.

ADDITIONAL THOUGHTS: YOU'VE GOT TO THINK FOR YOURSELVES!

From a translational cancer research perspective, and with respect to the data discussed in this overview, it is also important to consider experimental design with small molecule therapeutic agents when measuring and assessing cellular responses such as viability, autophagosome formation, and the degradation of HDAC and chaperones. Many of the cell biology effects discussed in this manuscript, using clinically relevant drug concentrations of neratinib, HDAC inhibitors, statins, and PDE5 inhibitors, are modest, with alterations of phosphorylation or expression being within a 50% reduction or a 2-fold increase.[25-27] Hence, it is possible that the *in vitro* data for neratinib, RAS, and YAP/TAZ using these physiologic concentrations may not induce enough of a biological alteration to actually kill tumours in a patient. On the other hand, using low clinically relevant drug concentrations for *in vitro* research is in stark contrast to the majority of cancer therapeutic manuscripts where much higher drug levels are used *in vitro* to observe effects with greater amplitudes, for example as described by Carrer et al.[69] and the concentrations of statins used in their work. Thus, before any laboratory-based study is performed, it is vital for investigators to determine from the literature/Phase I trials

the safe maximum plasma concentration and the area under the curve showing the plasma drug concentration over time. Usually, information is also provided by a drug company as to how much of their drug is protein-bound, probably inactive, and in the plasma. All of this information can be used to empirically judge at what concentration a drug should be used for cell-based studies, combined with other agents. For example, the maximum plasma concentration of sorafenib tosylate following a 400 mg ingestion is ~13 µM. However, sorafenib tosylate is >90% protein-bound in the plasma. Thus, for meaningful *in vitro* studies, the maximum drug concentration for cells growing in 10% (v/v) serum cannot realistically be >2 µM.[70,71] The obvious reasoning for this approach is that studies using a drug at a physiologic concentration, such as neratinib at 100 nM or below, may yield different biological information on viability, autophagy, and cell signalling processes versus studies using concentrations at an order or two above the safely achievable plasma drug level in a patient.

CONCLUSION: THAT RABBIT'S DYNAMITE

In conclusion, neratinib and derivatives of this drug are potentially of great importance in the fight against mutant RAS cancers. Neratinib as a single agent can act in carcinoma cells to simultaneously downregulate ERBB family receptors, associated quaternary complex receptors, and mutant RAS proteins in parallel with it. As a result, YAP phosphorylation and translocation of the co-transcription factor to the cytoplasm, this suggests that additional

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Optimised Tumour Sampling and Processing by a Multidisciplinary Approach for an Accurate Diagnosis in Non-Small Cell Lung Cancer

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Disclosure: The authors have declared no conflicts of interest.

Acknowledgements: The authors thank Angelo Casalini (University Hospital of Parma, Italy) and Michelangelo Fiorentino (University Hospital S. Orsola, Bologna, Italy) for their initial involvement and contribution to the discussion. The authors also thank Dr Luisa Granziero for providing editorial assistance on behalf of Health Publishing & Services Srl, which was funded by Novartis Farma SpA. Dr. Granziero declares there are no potential conflicts of interest relating to her assistance.

Support: This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Received: 04.05.19

Accepted: 02.07.19

Keywords: Cell-block, cytology, fine needle aspiration (FNA), histology, immunohistochemistry, next-generation sequencing (NGS), non-small cell lung cancer (NSCLC), tissue.

Citation: EMJ Oncol. 2019;7[1]:90-99.

Abstract

The classification of lung cancer has evolved parallel to the knowledge of its biomolecular features and is implemented by the analysis of specific gene alterations, which have shown prognostic and predictive values. Consequently, the diagnosis of a specific 'biomolecular subtype' of lung cancer is accompanied by different therapeutic strategies.

Optimal target tissue sampling plays a key role in the diagnosis and treatment of lung cancer. Tissue samples can be obtained through various techniques involving different healthcare professionals. Therefore, a multidisciplinary approach is crucial to obtain a suitable diagnostic sample encompassing as much of the information as possible for optimal therapeutic management. In this paper, the authors share the expertise of all professionals involved in the diagnostic and therapeutic approaches of patients with lung cancer: pulmonologists, pathologists, oncologists, radiologists, surgeons, and molecular biologists. The different know-how contributions have been gathered in a single text to offer a comprehensive view on the management of the lung cancer tissue journey.

INTRODUCTION

In the era of target and immunotherapy, the multidisciplinary approach of lung cancer is now mandatory. Close collaboration between pulmonologists, radiologists, surgeons, oncologists, pathologists, and molecular biologists allows a high success rate in the management of lung cancer patients. In this review, different healthcare professionals suggest practical recommendations in the optimised tumour sampling to improve the diagnosis and treatment of lung cancer patients in daily clinical practice.

THE ONCOLOGIST'S VIEW ON PREDICTIVE BIOMARKERS

Targeting oncogenic drivers promoting and sustaining tumour cells has transformed the care of patients with lung cancer. Biomarker-targeted therapies in advanced non-small cell lung cancer (NSCLC) had a 62% cumulative success rate, far higher than the 11% observed in absence of a biomarker-targeted indication.[1]

However, the precision medicine approach requires tissue samples and/or liquid biopsy to be collected at baseline to evaluate predictive markers, and at tumour progression to identify the mechanisms of resistance to therapy. The need for a new tissue sample from metastatic disease, with the associated risk, cost, and discomfort, may be motivated by the lack of sample from the primary tumour, specific requirements of the analysis (most RNA and phospho-protein tests require fresh frozen samples), and in some instances the clonal evolution of the tumour.[2,3] Sampling metastatic disease may only partially solve the problem of intratumour heterogeneity. Indeed, when many different clones are present in the patients' metastases, the predominance of a selected neoplastic clone may change with time, appear in different locations, and in regard to the most aggressive clone, not be represented in the core biopsy. Alternative sources, including circulating free DNA, circulating tumour cells (CTC), and circulating micro vessels and exosomes, can be considered.[4,5]

Multiplexed testing enlarged to *EGFR*, *ALK*, *ERBB2* (formerly *HER2*), *BRAF*, *PIK3CA*, *MET*, *NRAS*, *ROS1*, *NTRK*, *RET, NRG*, and other genes is fundamental to permit physicians to select therapies from first line of treatment and enrol patients in randomised trials.

Currently, testing a panel of predictive markers including *EGFR* and *BRAF* mutations, *ALK* and *ROS1* rearrangements, and programmed death-ligand 1 (PD-L1) expression is mandatory in advanced NSCLC to ensure the most appropriate first-line therapy. A useful and adequate algorithm has been developed, suggesting the simultaneous determination of *EGFR* and *BRAF* mutations; *ALK* and *ROS1* rearrangements; and PD-L1 expression in all advanced-stage non-squamous cell carcinomas, including never or former (<15 pack years) smokers with squamous cell carcinoma, and PD-L1 expression in advanced-stage squamous cell carcinomas (Figure 1). Following the approval of new promising biomarker-based drugs, a continuous updating of the algorithm will be required.[6]

THE RADIOLOGIST'S VIEW AND GUIDELINES FOR OPTIMISING BIOPTIC TARGETS AND IN RADIOLOGY

Indeterminate lung lesions are still a challenge for radiologists, oncologists, pulmonologists, thoracic surgeons, and pathologists. Lung tissue is obtained through minimally invasive techniques such as transbronchial biopsy/needle, trans-thoracic needle biopsy, endobronchial ultrasound-guided transbronchial needle aspiration (EBUS-TBNA), and endoscopic ultrasound-guided fine-needle aspiration (EUS-FNA) within or without the oesophagus and can also be done with an echo bronchoscope to perform histologic classification, with or without immunostains, and perhaps eventually with predictive biomarkers. Pre-procedural images allow technical planning including the selection of the most suitable imaging guidance, needle type, patient's position based on preferred access routes, and scheduled number of samples. No validated indications are currently available on the needle size for each patient or lesion.

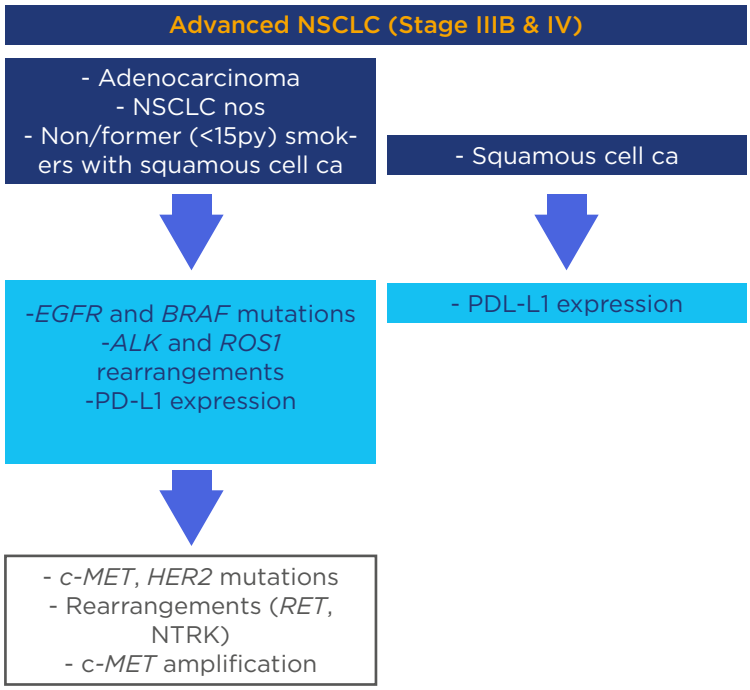

Figure 1: Clinico-pathologic algorithm describing the minimal requirements concerning predictive biomarkers in advanced non-small cell lung cancer starting with histology definition.
CA: carcinoma; EGFR: epidermal growth factor receptor; NOS: not otherwise specified; NSCLC: non-small cell lung cancer; PD-L1: programmed death-ligand 1.

While larger needles allow obtaining enough tissue for molecular tests, the needle size affects the risk of complications.[7]

Radiological diagnosis and staging of the lung lesions allow the appropriate selection of the patient referred to tissue sampling, the site of biopsy, and the bioptic technique (including the type of image guidance), provided that the images are carefully reviewed for the success of a biopsy, and that international, national, and institutional evidence-based guidelines are followed.[8,9]

In Italy, the Radiologic and Oncologic scientific societies (Italian Society of Radiology [SIRM] and Italian Association of Oncologic Medicine [AIOM], respectively)[9,10] provide original guidelines on lung cancer by endorsing recommendations from the main international societies (e.g., Fleischner Society, International Association for the Study of Lung Cancer [IASLC], etc.), even if multidisciplinary guidelines are still lacking. The sequential imaging approach, as recommended by the AIOM guidelines, includes chest X-ray, compared with previous radiological studies when available; followed by a contrast-enhanced CT of thorax, abdomen, and brain; and finally by PET-CT. The classification based on the new IASLC 8th edition tumour node metastasis (TNM) staging system for NSCLC is generally recommended.[11]

In particular, integrated contrast enhanced CT and PET-CT can accurately depict the tumour site and size, the invasion of the surrounding structures, the nodal involvement, and the metastatic sites, combining them in a TNM stage.[12] Based on this imaging and clinical staging (cTNM [including bronchoscopy and sometimes mediastinoscopy], thoracoscopy, EBUS-TBNA, EUS-FNA, and thoracentesis), each patient should be evaluated for curative surgical treatment by a multidisciplinary tumour board.

THE BRONCHOSCOPIST'S VIEW AND GUIDELINES FOR OPTIMISING BIOPTIC TARGETS

Several bronchoscopic biopsy techniques allow obtaining adequate cyto-histological samples for diagnosis and molecular characterisation of bronchial, pulmonary, and hilar-mediastinal-tumours. According to the location of the lesion, different approaches must be considered for sampling central endobronchial lesions, peripheral pulmonary lesions, and the pathological processes of the hilar-mediastinal area.

Central Endobronchial Tumours

Biopsy forceps are the most frequently used sampling instrument for endobronchial and submucosal lesions, with a sensitivity of 74–80%.[13,14] The limitation of biopsy forceps is the complexity of sampling submucosal and peri-bronchial lesions, and the risk of missing diagnostic tissue from lesions with a superficially large necrotic component. In these cases, TBNA and forceps biopsy in combination may significantly improve the diagnostic yield of bronchoscopy; however, the cost of TBNA discourages its routine use. Cryobiopsy showed significantly higher diagnostic yield (95.2%) compared with forceps biopsy (82.2%) in central lesions suspected of malignancy;[15] however, since it is an invasive procedure its use cannot be recommended routinely.

Peripheral Pulmonary Lesions

The diagnostic yield of bronchoscopic approach in these lesions is highly related to the size of the lesion, ranging from 5% to 64% for nodules <2 cm, to 30–75% for lesions >2 cm, and increasing to >80% for lesions with a diameter >4 cm.[16] In recent years, new technologies have been introduced in the clinical practice for the transbronchial biopsy of peripheral pulmonary lesions as endobronchial ultrasound radial mini probes, and electromagnetic navigation systems.[16] These new guidance technologies provide a higher diagnostic yield, especially for small lesions,[17] but they have higher cost compared to fluoroscopy.

Pathological Processes of the Hilar-Mediastinal Area

The only device available to obtain diagnostic samples from lesions of the hilar-mediastinal area is the transbronchial needle. TBNA demonstrated its efficacy both in the staging of

lung cancer and in the diagnosis of mediastinal pathology with a diagnostic yield of 78%.[18] The diffusion of EBUS-TBNA has allowed a great improvement in the diagnosis of hilar/mediastinal lesions, with a diagnostic sensitivity of >90%.[19] Furthermore, the combination of bronchial EUS-FNA with EBUS-TBNA allows a complete evaluation of the extent of the tumour by complementary access to different stations (nodes 8, 9, and 5).[20]

The Role of Rapid On-Site Cytological Evaluation in Optimising of Specimens

Rapid on-site cytological evaluation (ROSE) of cytologic smears is recommended as an assessment of adequacy of the specimens, regardless of sampling procedure. ROSE allows immediate quality assessment of the specimens collected during bronchoscopic procedures, especially transbronchial needle aspiration techniques. Based on this information, the operator determines whether additional material needs to be collected and assigned to further analyses required for target therapy setup. Although there is no evidence of increased sensitivity in TBNA or EBUS-TBNA by using ROSE, the immediate cytologic assessment reduced the number of needle passes and complication rates, and improves the adequacy of samples for molecular evaluation.[21]

THE THORACIC SURGEON'S VIEW AND GUIDELINES FOR OPTIMISING BIOPTIC TARGETS

Mediastinoscopy

Mediastinoscopy and video-assisted mediastinoscopy (VAM) are considered the reference standard for mediastinal staging of lung cancer, providing access to ipsilateral and contralateral superior mediastinal lymph nodes (stations 2L/R, 4L/R, and 7), with a sensitivity of 78% and 89%, respectively.[22] Surgical staging is indicated if negative results on EBUS/EUS are obtained and VAM is preferred to traditional mediastinoscopy.[23] Mediastinoscopy is very useful when a larger sample of tissue is needed for conclusive diagnosis and molecular profiling. Furthermore, VAM is advisable to provide histological information for small lymph nodes (<5 mm), or for restaging after neoadjuvant treatments.[23]

Video-Assisted Mediastinal Lymphadenectomy and Transcervical Extended Mediastinal Lymphadenectomy

Video-assisted mediastinal lymphadenectomy and transcervical extended mediastinal lymphadenectomy are invasive techniques that enable systematic lymph node dissection instead of sampling to improve the accuracy of transcervical surgical mediastinal staging.[24,25] These are not yet considered standard procedures and should be performed in very experienced centres because of their potentially higher morbidity (1.0–5.0%) and mortality (0.3–6.6%).

Video-Assisted Thoracoscopic Surgery

Video-assisted thoracoscopic surgery allows for the obtaining of large tissue samples of every mediastinal node station, and an accurate staging by exploring pleural space and performing pleural and lung biopsies. With a sensitivity of 96–100%, video-assisted thoracoscopic surgery can be considered a useful and safe alternative to traditional mediastinoscopy when it is contraindicated, not suitable, or not available, such as in the case of enlarged PET-positive para-aortic (station 6) and sub-aortic (station 5) nodes that are easily reached.[26]

THE PATHOLOGIST'S VIEW AND GUIDELINES FOR OPTIMISING TUMOUR TISSUE

The role of the pathologist is radically changed in the era of targetable oncogenes. The main barrier to attain a complete panel of biomarkers is accountable to the small amount of tissue available in small biopsy and cytology samples. Histology still has a role in selecting chemotherapy in PD-L1 negative and non-oncogenic driven NSCLC, and in guiding predictive molecular determinations. The increasing pressure for predictive biomarkers following first-line treatment will require larger

tumour tissue samples, and a correct handling of small biopsy and cytology to minimise failure rates.[27] Pathologists are called to improve and maximise the use of tumour tissue available by using several intra-laboratory approaches, such as the following:

Limiting Diagnostic Immunohistochemical Markers for Non-Small Cell Lung Cancer Subtyping

According to the World Health Organization (WHO) lung tumours classification,[28] morphology on haematoxylin-eosin (H&E) stain is sufficient to define histology in the vast majority of cases. Even in cases of a poorly differentiated NSCLC, it is important to restrict the immunostains at a minimum (TTF-1 and p40).[29]

Cell Block Preparation

Since various molecular biomarkers may be detected in lung cytologic specimens, the preparation of cell block (CB) is currently recommended. CB retains tissue architecture and provides multiple additional sections of various thickness for ancillary *in situ* or extractive analyses; moreover, CB may be archived. Different CB preparations are available with various yields. Plasma thrombin, direct clotting, and gel-based preparations are the most common, while collodion bags apparently give a higher cellular yield.[30] Formalin and alcohol are the most used fixatives.[31] When CB is prepared during rapid on-site evaluation, the first smeared slides (air-dried for Diff-Quick and alcohol fixed for Papanicolaou stains) should be used to state the adequacy of the presence and the amount of tumour cells, while all the remaining material should be dedicated to CB preparation. CB preparation may be obtained even from previous smears from FNA cytology, decolourising and removing the tumour cells, and then proceeding to CB preparation. This technique, called 'cytoscrape' may be performed even from archival material of NSCLC by gently scraping tumour cells off the slides to perform cellular clots.[32] CB provides a precious source of tumour cells; therefore, the entire volume from pleural or pericardial effusions should be submitted to centrifugation and have the pellet recovered, regardless of the number of molecular tests to be performed and the abundance of other available specimens.

Tumour Enrichment by Microdissection

To achieve high-quality molecular testing, the pathologist should mark the most suitable tumour area on the slide so that the optimal tumour content is extracted from cytology and paraffin-embedded material. Necrosis, bloody areas, mucous plugs, and inflammatory infiltrate should be eliminated from the selected material for fluorescent *in situ* hybridisation (FISH) analysis or extractive methodologies. Tumour cell microdissection attains a very high tumour/non-tumour cell ratio allowing the enrichment of tumour material. Moreover, the identification and selective isolation of tumour cells can be obtained from H&E stained slides examined under a light microscope. Laser-capture microdissection is an alternative method to perform tumour enrichment, albeit expensive and time-consuming. The ratio between tumour cells and non-tumour cells is of critical importance and, if possible, a minimum of 20–30% of tumour cells should be present in the material tested for genetic alterations to minimise false-negative results. Direct sequencing required at least 20–30% enrichment, and even a modern next-generation sequencing (NGS) approach may require at least 10% enrichment. Technologies based on real time PCR are needed for specimens with enrichment of 1–10%. Digital PCR may even allow analysis of specimens with tumour cell enrichment <1%, but its application is currently discouraged in tissue samples.

High Throughput Technologies

The introduction of NGS technologies in routine practice might permit a comprehensive characterisation of all current and next targetable genomic alterations (mutations and gene fusions) from relatively limited sources of tumour tissue,[33,34] therefore preventing the necessity to perform immunohistochemistry (IHC) and/or FISH. *EGFR* and *ALK* co-alterations were detected in up to 8% of cases using high-sensitivity peptide nucleic acid probe-based real-time PCR, ultra-deep NGS, and mutant-enriched NGS, with clinical

benefits.[35] Due to the ability of NGS techniques to detect hot spot mutations, copy number variations, and gene fusions at the same time, NGS platforms will likely replace the majority of other technological assets in molecular pathology. Several papers recently highlighted the efficiency and cost-effectiveness of NGS panels in simultaneously testing all predictive biomarkers,[36] possibly overriding the need for NSCLC subtyping.[37] Other high throughput methods are now available for the detection of multiple gene fusion transcripts, including the NanoString technology.[38]

Standardised Operative Procedures

After a minimised transfer time of tumour tissue from the operating rooms to the pathology laboratory, the samples should be immediately fixed in buffered formalin for 6–48 hours. To maximise tissue availability, it is important to perform minimally invasive sectioning during the preliminary diagnosis. Experienced technicians appropriately cut the paraffin block to expose complete surface of the bioptic fragments on the initial slides, without losing material for diagnostic ancillary techniques and molecular testing (microtomes equipped with 'waterfall' slides and 2–3 µm thick slides work perfectly for IHC). Since re-cuts should be kept at a minimum, another option is to cut multiple (approximately 20) unstained sections and keep them stored until a preliminary diagnosis has been made and ancillary testing is requested. This avoids tissue waste and shortens turnaround times (5–10 days), saving tissue and reducing costs.[39]

Multiple Blocks from Multiple Biopsies/ Single Biopsy

Separation of multiple fragments into multiple paraffin-embedded blocks may maximise the probability of successful molecular testing, consuming less tumour surface in a unique slide and saving tissue (separate blocks) for further molecular analysis, or material to submit for clinical trials.[33] Since PD-L1 expression is characterised by intratumoural heterogeneity, this determination should be tested along with the initial H&E-stained slide to cover the entire tumour surface, then the bioptic fragments should be separated into different blocks.[40] The authors strongly discourage alternative approaches using separate biopsies for routine practice and pathologist-uncontrolled molecular determinations.[41]

Management of Large Resected Samples

The optimisation of tissue sampling and handling can also be important for surgically resected samples from large tumours with heterogeneous features. In these cases, care should be taken in the fixation step, due to the slow penetration of formalin in tissue (5 mm per hour), as well as in tumour sampling, to accurately avoid necrotic areas. Moreover, considering the issue of tumour heterogeneity, the collection of different tumour areas in single paraffin blocks is advisable to maximise the detection of clinically important biomarkers in large tumours.

Reflex Testing

In eligible NSCLC patients, who do not receive the results of predictive biomarkers at their initial oncology consultation, the correct treatment is significantly delayed and frequently a second biopsy is performed to guarantee appropriate molecular testing after the initial consultation. About 19% of patients started chemotherapy before biomarker results became available. This can be avoided by incorporating reflex biomarker testing into diagnostic algorithms for NSCLC at the pathology level, and by further educating specialists to provide sufficient diagnostic cancer specimens for molecular testing.[39]

Tissue Decalcification

Decalcification procedures may complicate the results of biomarker determinations.[33] Decalcification irreversibly damages nucleic acids and prevents PCR-based molecular techniques as well as FISH. Therefore, bone biopsy should be avoided when the tumour target can be reached in a different site. It is good practice to warn the laboratory and address the bone biopsy directly to the pathologist involved in preparing the tumour tissue for molecular biomarker determination.[42]

To circumvent tissue decalcification, pathologists should separate calcified from fleshy material, processing the latter separately without decalcification. There are new available reagents for decalcification based on ammonia or ethy lenediaminetetraacetic acid that better preserve nucleic acids.

Different Determinations from a Single Sample

IHC and FISH analyses should be performed as a first step, exploiting the possibility to perform *EGFR* mutation analysis on the same sample previously submitted to *ALK* and/or PD-L1 IHC/FISH procedures, by scraping off tumour cells from stained slides.[39,43] Due to alcohol fixation, smeared cytology usually provides an excellent preservation of nucleic acids and smear areas highly enriched in tumour cells can be scraped for successful DNA extraction and sequencing.

The Minimal Amount of Tissue to Detect all Biomarkers in Routine Practice

The minimal amount of tumour tissue for adequate biomarkers testing depends on the type of genetic alteration to investigate, the sensitivity of the method, the type of tissue, and the laboratory expertise. *EGFR* mutations may be appropriately detected using a tumour/non-tumour tissue ratio >30% with ≥100 tumour cells. Detection of *ALK* rearrangement using FISH testing requires ≥50 tumour cells on biopsy or cytology, and ≥20 tumour cells are needed when *ALK* rearrangement is indirectly detected by means of IHC.[33,39,43] PD-L1 expression requires a minimum of 100 tumour cells.

The Molecular Biologist's View on ReBiopsy, Liquid Biopsy and Next-Generation Sequencing

Oncogene-driven and 'druggable' tumours undergoing pharmacologic resistance, for which an alternative therapy is available, require tissue rebiopsy, and all methods and guidelines described above play a major role.

Histologic change is a possible mechanism of drug resistance not only in various oncogene-driven settings (e.g., *EGFR* mutations, *ALK* rearrangement) and during immunotherapy, but also in wild-type NSCLC.[44]

Circulating tumour DNA (ctDNA) and CTC are important tumour-derived materials obtained from the bloodstream, which are a promising source of fresh material for detection of molecular biomarkers, for both diagnostic and monitoring purposes when tumour tissue is lacking. CTC, ctDNA, and other cancer-related material in the bloodstream are commonly known as 'liquid biopsy'. The technique is less invasive, repeatable, and rapid, possibly bypassing tumour heterogeneity.[45-47]

Studies comparing the sensitivity and specificity of ctDNA analysis to tissue biopsy in NSCLC confirmed the clinical value of this alternative approach.[48]

Recently, a valuable use of liquid biopsy has been proposed in the selection of metastatic *EGFR*-T790M-positive NSCLC patients, who have progressed on or after first-line therapy with *EGFR* tyrosine kinase inhibitors, eligible for treatment with osimertinib. The National Comprehensive Cancer Network (NCCN) guidelines reported that plasma genotyping may be considered at progression instead of at biopsy to define the eligibility for treatment with osimertinib; however, if plasma testing is negative, then tissue biopsy is recommended.[49] Availability of a potential blood-based test for ctDNA means that alternative biomaterials sources could be useful to select metastatic NSCLC patients sensitive to specific target therapy.

In the future, NGS-based liquid biopsy might represent an emerging approach in the treatment decision-making of metastatic lung cancer patients because it could ensure a comprehensive genomic profiling using biomaterial obtained through minimally invasive procedures (Figure 2). Moreover, NGS-based liquid biopsy could have a great advantage in overcoming spatial heterogeneity linked to tissue biopsy.[50]

At present, diagnosis and subtyping of lung cancer require a histology-based analysis; therefore, the co-ordinated use of tumour tissue from simultaneous sampling of conventional

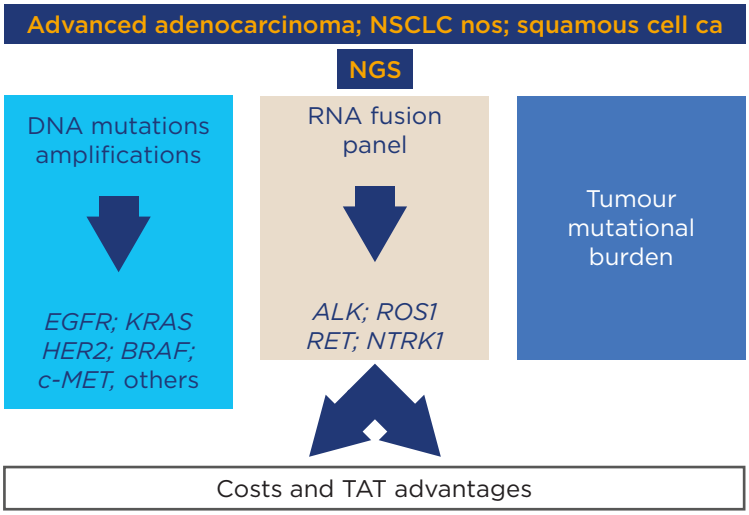

Figure 2: Next-generation sequencing including DNA and RNA analysis to simultaneously detect tumour cell mutations and rearrangements is a promising approach to perform multiple determinations using a limited amount of tumour tissue in a relatively short turnaround time and several cases.

CA: carcinoma; NGS: next-generation sequencing; NOS: not otherwise specified; NSCLC: non-small cell lung cancer; TAT: turnaround time.

biopsy/cytology and 'liquid biopsy' is the preferred approach.

CONCLUSION

Several molecular gene alterations have been unevenly identified in lung cancer, particularly in NSCLC with adenocarcinoma histology. The discovery of oncogenic drivers has led to the development of drugs tailored to the tumour molecular profile permitting a significantly higher clinical response and survival among biomarker-selected patients. Molecular-driven information allows innovative clinical trials and negates histology-dependent therapies. In this scenario, tissue biopsy still represents the gold standard for molecular analysis, but noninvasive or minimally invasive liquid biopsy methods are entering clinical practice, providing more tumour tissue for optimising molecular determinations and monitoring disease progression.

As exemplified in this comprehensive review, a close multidisciplinary collaboration among oncologists, pathologists, molecular biologists, radiologists, bronchoscopists, and thoracic surgeons is the only way to decide the best site to sample in obtaining more tumour tissue, limiting the procedural invasiveness, and furnishing the best neoplastic material for molecular analyses.

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Follow-Up of Accessory Breast Cancer Patient: Case Report

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Disclosure: The authors have declared no conflicts of interest.

Acknowledgements: The authors are grateful for histopathologist Dr Chowdhary Abdulhaleem for his valuable contribution.

Received: 24.04.2019

Accepted: 07.06.2019

Keywords: Accessory, auxiliary, breast cancer, ectopic.

Citation: EMJ Oncol. 2019;7[1]:100-106.

Abstract

Accessory breast is a congenital atavism condition. Accessory breast tissue may arise anywhere along the mammary line because of the failure of complete maturation during embryogenesis. The malignancy in accessory breast tissue is considered as primary breast cancer.

Axillary breast cancer is an under-recognised site of primary breast cancer. The authors presented a case report of a 52-year-old premenopausal female who presented with axillary immobile mass in her left axilla and who was diagnosed after extensive investigations with Stage II B oestrogen receptor (ER)/progesterone receptor (PR) positive, human epidermal growth factor 2/neu proto-oncogene (HER2/neu) negative, and poorly differentiated accessory breast adenocarcinoma. The patient was designated as Stage II B, and following the 2012 National Comprehensive Cancer Network (NCCN) guidelines for breast cancer management, was surgically treated, followed by postoperative adjuvant chemotherapy in the form of four cycles of doxorubicin and cyclophosphamide (AC protocol), and then four cycles of docetaxel. Subsequently, radiotherapy was given followed by hormone therapy. The patient was followed up for 7 years, and at the time of publication, is alive and stable.

Accessory breast cancer is a rare disease and misdiagnosis of these cases is a common problem, leading to extensive and unnecessary investigations; therefore, physicians must be aware of these cases. Management of accessory breast cancer is according to the same guidelines provided for management of the condition. Follow-up data should extensively encourage the determination of the prognosis of accessory breast cancer in comparison to common breast cancer.

INTRODUCTION

Accessory breast cancer is a rare disease developed from accessory breast tissue. Accessory breast tissue can be found along any point of the mammary lines, including in the thoracic and abdominal region (67%) and as low as the groin. Ectopic breast tissue can also be found in locations such as the face, back, and thighs, but the predominant site is the axilla. The

incidence of supernumerary breast and ectopic breast tissue around the world is 1–6%.[1] It affects 2–6% of females and 1–3% of males, but there is no definite incidence number for accessory breast malignancy; furthermore, reports for accessory breast cancer only include case reports and case series. Occurrence rates differ extensively according to ethnicity and gender, ranging from as low as 0.6% in Caucasians (relatively common among Asian women) to as high as 5% in Japanese females and Native American populations.[2,3] The 1915 Kajava classification system classified accessory breasts as Class I–VIII according to anatomical structure, and is still used today (Table 1).[4,5]

Accessory breast tissue can be located along the chest wall, vulva, axilla, knee, lateral thigh, buttocks, face, ear, and neck. Changes or symptoms may be noticed during puberty, at different times of the menstrual cycle, or during pregnancy and breastfeeding in women. Of note, the accessory breast tissue is often not detected until puberty because it is activated by hormones.[6]

CASE REPORT

A 52-year-old premenopausal female with diabetes, a blood pressure of 130/70 mmHg, a weight of 76 kg, a height of 167 cm, and no family history of breast cancer presented to the general surgery outpatient department on the 4th of December 2011. The patient had a history of left axillary immobile mass of around 3.0x2.0 cm (length/width) which had increased in size within the prior 6 months, but had no history of a lump in the breast or discharge from the nipple. The patient mentioned that this axillary mass had been present since puberty but had been gradually increasing in size. The patient had a negative family history of malignancy and ultrasound (US) breast screening was performed which showed no mass in the breast. Multiple lymph nodes were seen, however, in the left axilla, with the largest lymph node being 4.4x3.5 cm.

On the 17th of December 2011, tissue biopsy was taken from the left axillary mass which measured 4.5 cm in maximum diameter, weighed

Table 1: Kajava classification 1915.

Classes	Description			
	Glandular tissue	Nipple	Areola	Comment
Class I	☑	☑	☑	Complete breast
Class II	☑	☑	☒	
Class III	☑	☒	☑	
Class IV	☑	☒	☒	
Class V	☒	☑	☑	
Class VI	☒	☑	☒	
Class VII	☒	☒	☑	
Class VIII	☒	☒	☒	Hair

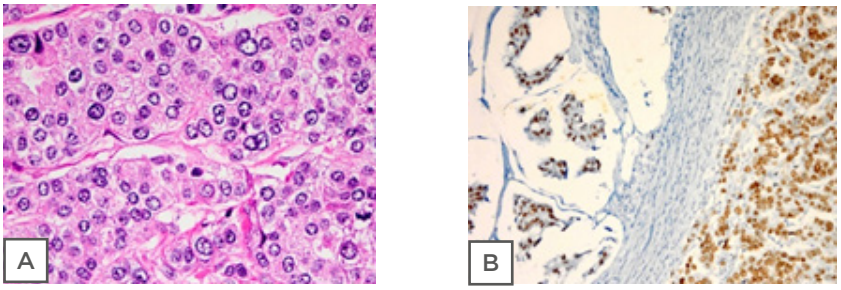

Figure 1: A) Histological examination of specimen. Sections show large deposits of carcinoma replacing most of the lymph node structure. B) The tumour cells in both mucinous and nonmucinous areas show a positive staining for oestrogen receptor.

30 g, and to which bio-section showed a greyish-white solid cut surface with pinkish and yellowish areas. Four blocks representing the whole cross-section of the tumour were embedded in three cassettes. Microscopically, the sections showed lobules of a malignant epithelial tumour involving the subcutaneous adipose tissue, dermis, and reaching up to the overlying epidermis. It showed large areas of partial or complete ischaemic necrosis, scattered mitotic figures, and tumour cells arranged in solid sheets or lobules without glandular differentiation (Figure 1A). Immunohistochemistry (IHC) methodology reported that the tumour cells stained positively for pan-cytokeratins (CK) (AE1/AE3), suggestive of lymph node micrometastases;[7] CK7; epithelial membrane antigen; ER, that formed 50% of tumour cells; and PR, that formed 5% of tumour cells (Figure 1B).

Table 2: Immunohistochemistry test results.

Test	Reaction	Comment
CK AE/1/3	✓	Suggestive of lymph node micrometastases.
CK7	✓	Oestrogen receptor (that formed 50% of tumour cells) and progesterone receptor (that formed 5% of tumour cells).
EMA	✓	Epithelial membrane antigen.
HER-2	Negative	Human epidermal growth factor receptor 2.
BerEP4	Negative	Histologic stain used to aid in the diagnosis of basal cell carcinoma.
Mammoglobulin	Negative	Highly specific for most breast cancers.
CK5/6	Negative	Cytokeratin 5/6: help to differentiate between mesothelioma and other forms of cancer.
TTF-1	Negative	Thyroid transcription factor-1: sensitive marker for pulmonary and thyroid adenocarcinomas.
CD10	Negative	Sensitive immunohistochemically marker of normal endometrial stroma.
SMA	Negative	Smooth muscle actin aid in diagnosis of ovarian carcinoma.
S100	Negative	Common marker of neural tissue/ lesions and melanoma.
CgA	Negative	Chromogranin help diagnose carcinoid tumours.
Synaptophysin	Negative	Common neuroendocrine marker.
CK20	Negative	Cytokeratin 20.
p63	Negative	(Rule out invasion in breast tumours by determining presence of myoepithelial cells).
p53	Negative	Tumour marker in early stages of lung, skin, head and neck, and oesophageal cancer.
(CA 15-3)	High 107.7	Tumour marker for breast cancer.
CA 125	Normal	Tumour marker for breast cancer.

IHC results were not exclusively positive; HER2, BerEP4 (histologic stain used to aid in the diagnosis of basal cell carcinoma), mammoglobulin (highly specific for most breast cancers), CK5/6 (aid to differentiate between mesothelioma and other forms of cancer), thyroid transcription factor-1 (sensitive marker for pulmonary and thyroid adenocarcinomas), CD10 (sensitive immunohistochemical marker of normal endometrial stroma), smooth muscle actin (aid in diagnosis of ovarian carcinoma), S100 (common marker of neural tissue/lesions and melanoma), chromogranin (aid in diagnosis of carcinoid tumours), synaptophysin (neuroendocrine marker), CK20, p63 (rules out invasion in breast tumours by determining presence of myoepithelial cells), and p53 all showed a negative reaction. Therefore, the possibilities of neuroendocrine, primary lung, primary renal tumours, among others were not supported by the IHC results. Reports showed moderate to poorly differentiated adenocarcinoma in favour of primary breast cancer (Table 2).

On the 26th February 2012, the patient was referred to medical oncologists for further management and workup. Chest, abdomen, and pelvis CT scans were performed to rule out any primary sites, which all came back negative, and only the left axillary lymph node was seen to be involved. Bone scan results, as well as upper gastrointestinal endoscopy and colonoscopy results investigating for primary tumours, were also negative, and there were no signs of any malignancy. MRI of the breast revealed no pathology apart from multiple axillary lymph nodes. Tumour markers; cancer antigen-breast (CA 15-3) was high at 107.7 and CA 125 was normal (Table 2).

In April 2012, she underwent left axillary clearance and the histopathology report confirmed primary breast adenocarcinoma.

On the 19th of May 2012, the patient received chemotherapy; AC protocol doxorubicin 60 mg/m^2 and cyclophosphamide 600 mg/m^2 every 21 days for 4 cycles, followed by 4 cycles of docetaxel 100 mg/m^2. Every cycle was 21 days and the patient tolerated it well. The patient then underwent radiotherapy and hormonal therapy (tamoxifen 20 mg oral daily). She underwent follow-up every 6 months until present. To assess the prognosis of the case, the Nottingham prognostic index for breast cancer was used. It gave an index value of 4.6, placing it in the moderate group, with 5 year survival.[8] The National Health Service (NHS) Predict tool was applied as well for prognostic assessment, including hormonal status, and showed a result of 10 years survival.[9] In 2019, after 7 years of starting the treatment, the patient is doing well, free from any signs of malignancy, recurrence, or complications. The mammography (MMG) results were negative.

DISCUSSION

Accessory breast is a congenital condition when accessory normal breast tissue is present at abnormal sites.[10] Accessory breast is also known as polymastia, supernumerary breasts, auxiliary breast, ectopic breast, adnexal, or mammae erraticae. These types of breasts sometimes appear to have or lack nipples or areolae, making them ambiguous.[11]

In Caucasians, the incidence of ectopic breast tissue is 1–4%, while it is more frequent in the Far East, especially Japan, among both genders. Its incidence rate was evaluated as 5.19% in Japanese women, and as 1.68% in Japanese men with hereditary factor.[12] These abnormal tissues are most frequently seen in the axilla, followed by the area just lower and in half of the cases this abnormality is shown on both sides.[12] In rare cases it was reported in some other areas including the acromial or scapular region, vulva, and in the midline of the thorax and abdomen.[13] Ectopic breasts enlarge during pregnancy and lactation and may lactate if they have a working ductal system. Breastfeeding from ectopic breasts was reported.[12] Disorders of breast tissue such as adenofibroma, cysts, and carcinomas have also been reported in ectopic breasts similar to normal breast tissue and carcinomas are rare.[14-16] The types of carcinoma seen within the ectopic breast tissue include ductal, lobular, mucinous, medullary, papillary, and invasive secretory (juvenile) carcinomas.[13,15,16]

Ectopic breast tissue develops embryologically because of failed resolution of the mammary ridge or milk line. An ectodermal tissue extends from the axilla to the inguinal folds and shows in the 6th week of gestation. The axilla is the most

frequent site followed by the area inferior to the normal breast and may appear anywhere along the milk line.[17]

Hormonal influences affect ectopic breast tissue, just as they would in a healthy breast, and can develop similar types of benign and malignant disorders. Fibroadenomas, cysts, duct hyperplasia, and infrequently carcinoma can arise.[18,19] The incidence of accessory breast cancer as per reports is between 0.3% and 0.6% of all breast cancers.[19]

Ectopic primary breast cancer in the axilla comprises 60–70% of all cases reported.[20] Differential diagnosis of the accessory breast cancer includes many disorders. In the axillary area, it can be confused with lipoma, lymphoma, lymphadenitis, metastatic lymphadenopathy, sebaceous cyst, and hidradenitis suppurativa. MMG and US of the breast can aid the exclusion of other breast pathologies. US can also detect ectopic breast tissue as an echogenic area resembling normal glandular tissue, as well as detect characteristics of the mass. MMG cannot capture ectopic breasts because of their peculiar location, but it can be visualised in the axilla by oblique and exaggerated craniocaudal views.[21]

Cancer of ectopic breasts is depicted as a typical malignant mass with the same characteristics of those of metastatic axillary lymph nodes associated with malignant tumour. No specific findings for accessory breast cancer are detected.[21] Using MRI, the signal intensity of ectopic breast tissue is similar to that of the adjacent breast tissue, but with variability of the amount of interspersed fat. Pathological confirmation through fine-needle aspiration cytology or Tru-cut® biopsy of the mass should be performed to harvest suspect cells or tissue. Invasive ductal carcinoma, such as in traditional breast cancer, is the most common histological type with 79% of all accessory breast cancer.[22]

Lobular, mucinous, medullary, apocrine, and papillary carcinomas are detected in these cases and cystosarcoma phyllodes are also described. In 2011, Nihon-Yanagi et al.[20] found that medullary, mucinous, and apocrine carcinomas were more common among accessory breast cancer for unknown reasons.

Regarding the management, surgical interference of accessory breast cancer combines wide resection of the tumour with surrounding tissue, skin, and axillary lymph nodes dissection.[18] Ipsilateral mastectomy has no additional benefit regarding survival considering that MMG and US of the anatomic breast are normal, as was in the present case, but should be performed when differential diagnosis is challenging in some situations.[18]

In 2015, Zhang et al.[18] recommended mastectomy if the accessory breast is closely connected to normal breast tissue, otherwise it is unnecessary.

The same regimens and protocols for postoperative treatment are used for anatomic breast carcinoma. Radiotherapy of the tumour site is recommended to control local spread, and radiation of the ipsilateral anatomic breast is not usually performed. Adjuvant therapy is mostly required because lymph node disease is usually also present. The prognosis of accessory breast cancer is difficult to assess due to limited follow-up and staging data as well as small sample size. Some authors have reported worse outcomes of accessory breast cancer than other anatomic breast cancer as the tumour is near the axillary lymph nodes and is therefore exposed to early metastasis to these nodes.[18]

In a report in 2011, 94 Japanese cases were reviewed and indicated that accessory breast cancer has a higher risk of lymph node metastasis than usual breast cancer.[20]

Accessory breast tissue is more disposed to malignant change than normal breast tissue. No definite number is reported for the incidence of accessory breast cancer among population.[23] Accessory breast cancer patients experience clinical presentations as swelling, thickening, tenderness, irritation, and sometimes limited motion of shoulder. These symptoms are commonly exacerbated at the onset of puberty and pregnancy.[24] Accessory breast cancer is a rare entity and has a substantial chance for misdiagnosis especially when there is an absence of anatomical breast structure such as the nipple and areola.[25] The accessory breast may also not show up; therefore, MRI can be used to differentiate it from the normal breast tissue.[3]

The diagnosis as well as symptoms of accessory breast carcinoma are the same as for breast

carcinoma,[16] except for some differences including excess axillary fat, lymphadenitis, lymphoma, metastatic carcinoma, and hidradenitis suppurativa.[6] Accessory breast cancer is diagnosed as breast carcinoma using MMG and US, followed by pathologic diagnosis by fine-needle aspiration cytology or core biopsy.[26]

The MMG is an effective tool for the assessment of breast carcinoma but not for accessory breast cancer assessment. Core tissue biopsy especially when accompanied by IHC is a very effective tool for early diagnosis and treatment of accessory breast carcinoma.[2]

The presented case was very perplexing because it was a rare condition for primary axillary breast cancer. The lack of areola and nipple (according to Kajava classification 1915 this case classified as Class IV) made the clinical diagnosis very difficult, confounded by the IHC results being inconclusive. This lead to extensive and unnecessary lab tests and investigations. The possibility of the normal breast being the primary site was not supported by negative staining of mammoglobulin, gross cystic disease fluid protein. Furthermore, IHC did not support the possibilities of a neuroendocrine tumour or the lung, and the kidney as the primary sites.

In the beginning the case was suspected to be a metastatic adenocarcinoma with small components of mucinous carcinoma; however, the lymph nodes were positive (13/15) with large deposits of carcinoma replacing most of the lymph node structure favouring primary breast including the possibility of axillary (accessory) primary breast. The possibilities included breast, and to a lesser extent, primary adnexal tumour. Oncologists were not satisfied about histopathology results, and so all thoughts were redirected towards it being a secondary tumour from the breast or other sites. As such, more investigations were requested including CT, MRI, CA 125, and upper gastrointestinal endoscopy, but all results were negative for the detection of unknown primary tumours.

A Tumour board committee was held to discuss this case and it was agreed that it is a primary accessory breast tumour because no original tumour could be detected in the breast, adnexa, colon, or lung. The histopathology was in favour of primary breast because CA 15-3 high and ER and PR were positive as well as previous case reports and literature review for similar cases.

The patient was staged as Stage IIB, ER/PR positive, and HER2/neu negative poorly differentiated adenocarcinoma.

The patient was treated with common breast carcinoma methods following NCCN guideline 2012[27] by surgery followed by chemotherapy. This included 4 cycles of AC and 4 cycles of docetaxel followed by a 50 g radiotherapy and tamoxifen for 5 years, and cancer antigen (CA 15-3) showed a significant decline after treatment (Figure 1).

Prognosis of accessory breast cancer is difficult to institute because of the absence or limited follow-up data as well as the small sample size,[28] in the follow-up case the patient is stable with very good performance status for 7 years and without any complications.

CONCLUSION

Accessory breast cancer is an uncommon type of cancer and the incidence of it has no definite number. The diagnosis of these cases is challenging, and misdiagnosis will lead to extensive and unnecessary investigations. Despite the fact that carcinoma arising in axilla as a primary site is a rare condition, still the possibility of accessory breast cancer should still be considered. Management of accessory breast cancer should follow the same guidelines for breast cancer. It is important to encourage follow-up data for these cases to establish the prognosis of accessory breast cancer comparing breast carcinoma.

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