Novel technologies in the treatment and monitoring of advanced and relapsed epithelial ovarian cancer

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Abstract

Epithelial ovarian cancer (EOC) is the fifth most common cause of cancer death in females in the UK. It has long been recognized to be a set of heterogeneous diseases, with high grade serous being the most common subtype. The majority of patients with EOC present at an advanced stage (FIGO III–IV), and have the largest risk for disease recurrence from which a high percentage will develop resistance to chemotherapy. Despite continual advances in diagnostics, imaging, surgery and treatment of EOC, there has been little variation in the survival rates for patients with EOC. In this review we will introduce novel bioengineering advances in modelling the lymphatic system and real-time tissue monitoring to improve the clinical and therapeutic outcome for patients with EOC. We discuss the advent of the non-invasive 'liquid biopsy' in the surveillance of patients undergoing treatment and follow-up. Finally, we present new bioengineering advances for palliative care of patients to lessen symptoms of patients with ascites and improve quality of life.

Introduction

Epithelial ovarian cancer (EOC) is the second most common genital malignancy after uterine cancer in women and accounts for the majority of deaths from gynaecological malignancies in western countries. Lifetime risk is approximately 1.6%; the latest data show that 1 in 43 women will develop EOC during their lifetime. Women with a mutated BRCA1 or BRCA2 gene are at increased risk ranging between 25% and 60% depending on the specific mutation [1].
There is increasing clinical and molecular evidence in support of the fallopian tube epithelium being the source of the cell of origin of high-grade serous ovar- ian cancer [2]. This was derived from the detection of premalignant cells in the epithelium of the fallopian tube known as serous tubal intraepithelial carcino- mas (STIC) in patients with BRCA 1 or 2 mutations in risk reducing salpingoophorectomy specimens. STICs are considered as the most likely precursors of the high grade serous ovarian cancer (HGSOC), which is the most common histological sub-type in patients with EOC [3]. The underlying hypothesis is that ovar- ian cancers may actually be of tubal origin after 'drop

metastases' of cancer cells on the ovarian surface or after dislocation of transformed fallopian tube epithe- lial complexes at ovulation onto the ovarian cortex. In both states, the disease appears to have started in the ovary, whereas it is now becoming evident that it is actually initiated in the fallopian tube. This tubal car- cinogenesis most probably occurs in a stepwise man- ner. Initiated from a non-proliferating 'p53 signature', serous intra-epithelial carcinoma develops through a 'proliferating p53 signature'. The development of HGSOC from a tubal precursor lesion cannot be his- topathologically established in 40–80% of the cases of advanced cancer, particularly when there is no BRCA 1/2 mutation present. Therefore, an independent coex- istence without a direct association of the two lesions is possible. It is likely that not all HGSOCs originate in the fallopian tube, but that many parallel developmental pathways exist that have not been fully established yet. As a result of this, risk-reducing surgery is incapable of preventing 100% of all future invasive EOCs [3].

Despite the continuous advances in diagnostics and imaging, more than 70% of the patients with newly diagnosed EOC will present with an advanced stage FIGO III and IV. This is mainly attributed to the unusual tumour biology and clinical behaviour of the disease, which is typically associated with locoregional dissemination throughout the peritoneal cavity result- ing in symptoms which only present at a later stage in a rather unspecific symptoms pattern, including abdominal bloating and distention with pain, urinary frequency, postmenopausal bleeding, loss of appetite and occasionally rectal bleeding [4]. This unusual natu- ral history has therefore generated unique therapeutic strategies that clarify the important contribution of locoregional control to survival for this disease.
The last decades have brought a significant advance in the treatment of EOC, both in surgical and systemic aspects, with the development and addition to stand- ard treatment of extensive cytoreductive techniques, refinement of surgical skills in the upper abdomen, dose dense regimes and novel targeted therapies. Neverthe- less, the survival rate of women with EOC has changed little since the revolutionary platinum based treatment that was introduced more than 30 years ago [5, 6].
Only in recent years, targeted therapies based on the principle of anti-angiogenesis and homologous recombination repair mechanisms have brought a sig- nificant efficacy in the treatment of EOC; bevacizumab, pazopanib and olaparib have proven, in a maintenance regime during and/or after successful chemotherapy, their efficacy in significantly prolonging progression free survival (PFS), but failed to significantly influence the overall survival (OS) of the patients [7]. Possible mechanisms discussed for this consistent discrepancy is the high rate of cross over in the subsequent lines that impurify any survival benefit attributed to each agent. Despite all the advances brought by novel targeted agents, in advanced disease, one of the strongest predic- tors of survival remains the amount of residual tumour after debulking surgery [8]. Therefore maximal effort surgery aiming at maximal tumour reduction, ideally without any macroscopic tumour residual disease, fol- lowed by adjuvant systemic chemotherapy typically with paclitaxel and carboplatin, constitutes the cur- rently established gold-standard in the primary man- agement of the disease [8]. The hypothesis underlying the value of surgery is mainly based on the removal of 'bulks' of tumour so EOC is more responsive to systemic chemotherapy. This enhancement of response to cyto- toxic treatment may theoretically be achieved through the reduction of tumour mass critical for development of second resistance by minimizing tumour areas with poor perfusion and resecting of primary resistant tumour clones. These assumed mechanisms are derived from the hypothesis that platinum-resistant clones are generated by clonal diversity from the outset of the dis- ease and co-exist in the chemo-naïve state, represent- ing a slowly growing chemo-resistant 'second disease' that eventually recurs clinically well after the dominant presenting clone has been thoroughly controlled [9]. This complex interaction of tumour biology and surgi- cal effort could be mathematically projected as a result of tumour volume by time as source of development of

chemotherapy resistance. Validated data show that in the primary presentation of the disease, for each 10% increase in cytoreduction there is a direct correlation to a 5.5% increase in median survival of those patient populations [10]. Increasing surgical effort, continuous education and practice and growing expertise seem to clearly be associated with improved rates of primary cytoreduction with no incremental increase in opera- tive morbidity [11–14].

Nevertheless, despite this maximal effort in every level, patients' outcomes vary broadly in terms of surgi- cal and clinical aspects. So far there are no valid preop- erative biomarkers established that can reliably predict surgical and clinical outcome, so that surgical approach cannot currently be individualized and tailored to each patients' needs and tumour profile. This leads poten- tially to major morbidity without the equivalent thera- peutic benefit. We know for example that despite total macroscopic tumour clearance 20% of the patients will relapse within the first 12 months after surgery [8]. Therefore, these patients would need to be directed towards alternative treatment strategies.

In the present review we aim to give an overview of the most recent bioengineering advances that may be implemented to overcome clinical and therapeutic dilemmas in advanced and relapsed EOC and improve surgical outcomes by better patient stratification and selection of ideal surgical candidates. We will also pre- sent some bioengineering approaches for the palliative situation of the disease to alleviate the symptoms of patients with end stage ovarian cancer and ascites.

Tumour dissemination patterns and surgical outcome at primary surgery

An identification of the tumour dissemination patterns followed in the primary and subsequently in the recurrent situation of EOC is essential for the better understanding of the disease in order to enhance the evolution and refinement of all therapeutic approaches. EOC is however strongly heterogeneous and hence constitutes a major challenge to the success of therapy regimes. Initial attempts based on the profiling of single-tumour biopsies at presentation failed to develop personalised-medicine strategies. Understanding the heterogeneity is therefore a major aim en route in identifying signatures that can indicate treatment failure and subsequent relapse. The level of heterogeneity is not only spatial but also temporal. A systematic comparison of the intraoperative tumour dissemination patterns and surgical outcome of women who underwent both primary and secondary tumour debulking surgery in the same institution within a 10 year period of time demonstrated that a different tumour 'behaviour' appears to be followed in the primary compared to recurrent situation of the disease even at the same patient, while interestingly the primary tumour patterns do not appear to have any predictive value for the tumour patterns at recurrence[15]. Venturing even beyond surgical borders, one could therefore assume that ovarian cancer reappears under a different dissemination profile than at its initial presentation with a higher tendency for dissemination and highly probable different tumour biological profile. Therefore, novel biomarkers are warranted in order to predict tumour patterns followed at recurrence and hence optimize treatment.

Lymphatic transport analysis

Next to peritoneal tumour dissemination pathways, the other important metastatic route of advanced EOC is the lymphogenic pathway. At least 50% of the patients with advanced disease will present with positive retroperitoneal pelvic and/or paraaortic lymph nodes [16]. The routes of lymphogenic dissemination are not well defined and the impact of lymphadenectomy cannot be predicted in terms of long term morbidity such as lymphoedema etc. Despite the importance of lymph nodes in ovarian cancer, very little is known about how immune cells, cancer cells, antigens and lymph move through nodes and interact. There are scant data on the time scales for antigen uptake by B-cells, for example, and there is a distinct lack of modelling that would facilitate the testing of new hypotheses, performing virtual 'experiments' that could not be done *in vivo*, and the development of better treatment modalities such as replacement node constructs. Recently, computational modelling has been applied to elucidate patterns of lymph flow through nodes [17, 18]. These studies revealed that only a small percentage of lymph flows through the paracortex. Further physiologic models can make use of recent multi-photon imaging of mouse lymph nodes, as well as histological observations on human lymph nodes gathered lymphadenectomies during primary debulking surgery. Well-constructed models have the power to contribute significantly to the knowledge base on immune reactions, cancer cell invasion, as well as the characteristic swelling that can result from these phenomena. The resulting information will hopefully provide unprecedented insight into lymph node function that will potentially benefit great numbers of cancer patients [17].

The transport of lymph, cells, and antigens within the lymphatic vessels is a key component of lymphatic and immune functions and numerous pathologies. In the absence of such transport, antigen encounters and immune cell propagation of information would be limited to cell and diffusion scale limits of at most hun- dreds of microns. Under pathological conditions, this system undergoes tremendous remodelling, includ- ing fluid volume shifts and immune cell recruitment. Lymph node swelling starts within hours of an immune challenge and is likely due to some combination of hyperplasia and fluid shifts. Hyperplasia may occur in the follicular zones, paracortical zones or sinus due to infections, autoimmune disorders, inflammatory lesions or malignancies but take many tens of hours to

occur. Better knowledge of lymph node transport and the environment in which immune cells operate could aid efforts to limit metastatic spread of cancers, improve vaccines, and design lymphatic tissue constructs [19].

Real-time tissue monitoring

Monitoring human tissue by measuring levels of biomarkers in the extracellular fluid is becoming an increasingly important approach to provide the clinical care team with an assessment of the tissue state and of how the tissue state changes with disease progression or treatment [20]. Using this approach, the molecular communication between neighbouring cells or molecular exchanges between cells and the local blood supply give a moment-by-moment picture of tissue function. Historically this 'sampling' of the tissue was achieved by measuring levels in single blood or urine samples. If we want to record levels continuously a different approach must be used.

One way to do this is to implant a biosensor or meas- urement device into the tissue. This has been achieved to some degree for continuous glucose monitoring for diabetics [21] and for tissue oxygen measurements (for example by pulse oximetry). Beyond this it is challeng- ing at a research stage to develop devices. Systems using miniaturized NMR probes combined with targeted magnetic nanoparticles to detect and profile cancer cells have been shown [22]. An alternative approach is to use techniques such as the iKnife, where the 'smoke' released during electrosurgical dissection of the tissue is injected directly into a mass spectrometer [23].

An alternative approach is to use a sampling device. Microdialysis involves placement of a small probe into the tissue of interest. The probe has a semi-permeable membrane at its tip, which is perfused by a microfluidic flow system. Biomarkers in the tissue diffuse through the membrane and are carried out of the tissue by the flow, as demonstrated in figure 1. Continuous real-time analysis can then take place in a controlled environ- ment, for example at the patient bedside.

Microdialysis monitoring of tissue metabolism

Human tissue has a complex relationship to local blood supply, with its metabolic state reflected in a tight balance between local activity and blood supply. Real- time tissue measurements allow detection of transient changes in the metabolic state of the tissue such as hypoxia and ischaemia. Online microdialysis allows continuous sampling of tissue metabolites and can be coupled to an online system to provide high temporal resolution, allowing detection of dynamic changes [24–26]. Microdialysis has been used extensively in combination with online analysis systems to detect tissue health and ischaemia [27–31].

An example of human tissue monitoring where there is a clear ischaemic event is free flap surgery. In figure 2, a radial forearm flap is monitored as it raised, disconnected from the blood supply and repositioned for maxillofa- cial reconstructive surgery following resection of an oral

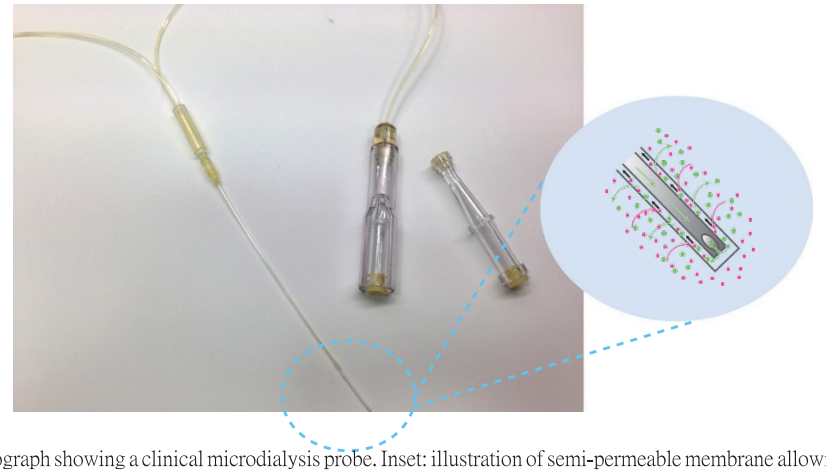

Figure 1. Photograph showing a clinical microdialysis probe. Inset: illustration of semi-permeable membrane allowing exchange of molecules between the tissue and the probe lumen.

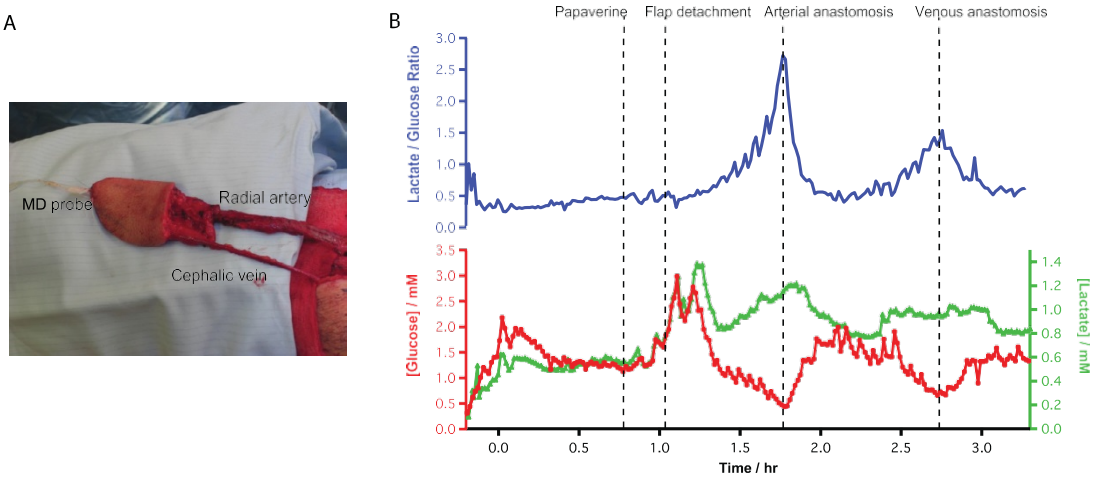

Figure 2. (A) Photograph showing a microdialysis probe inserted into a radial forearm flap prior to detachment from the blood supply. (B) Dialysate metabolite levels during free flap surgery [30]. The bottom graph shows dialysate glucose (red) and lactate (green) concentrations (green) and the top trace (blue) shows the corresponding lactate/glucose ratio. The dotted lines indicate key events during the surgery. A topical vasodilator, papaverine, was applied to the tissue, which caused a transient increase in both glucose and lactate, although this was not observed in the lactate/glucose ratio and was therefore most likely due to increased blood flow. Upon cessation of blood flow, glucose levels decreased and lactate levels increased, indicating the onset of ischaemia. This can also be seen in the increasing lactate/glucose ratio upon detachment of the flap. Following successful arterial anastomosis, dialysate glucose levels increased, lactate levels decreased and the lactate/glucose ratio decreased, indicating that the ischaemia had been alleviated. The final stage involved re-clamping the vessels while the venous anastomosis was carried out. At this point, the metabolite levels indicated the onset of ischaemia, with levels returning to normal once blood flow was re-established. Reproduced with permission from [30]. CC BY 4.0.

tumour. The metabolic changes are shown most clearly in the lactate/glucose ratio, which rises until anastomosis re-established local blood flow in the new site [30].

Such an approach can also be used to monitor can- cer tissue, as shown in figure 3. Tissue with high tumour burden has a different relationship with local blood flow compared to tumour-free tissue. Tumour cores are often hypoxic, and most tumour cells rely on aerobic glycolysis rather than mitochondrial oxidative phos- phorylation to generate energy. Careful choice of probe characteristics will enable measurement of much larger biomarkers associated with inflammation, tissue infil- tration and disease progression. For instance, matrix metalloproteinase 2 has been measured dynamically in the human heart during cardiac surgery [32].

Microdialysis drug delivery and tracking

There has been considerable interest recently in engineering devices that allow local delivery of drugs directly to the tissue providing a personalized treatment approach for each patient [33]. Examples have been shown in which drug effectiveness is determined using histology and possibly followed up with systemic drug administration [34, 35]. As an alternative approach, recent work within the Boutelle group has proposed carrying out retrodialysis using a microdialysis probe implanted in the tumour to locally deliver chemotherapy drugs such as carboplatin [36]. In order to monitor the drug dosage a second microdialysis probe would be inserted into the surrounding healthy tissue to monitor the first arrival of the drug in real time. An illustration

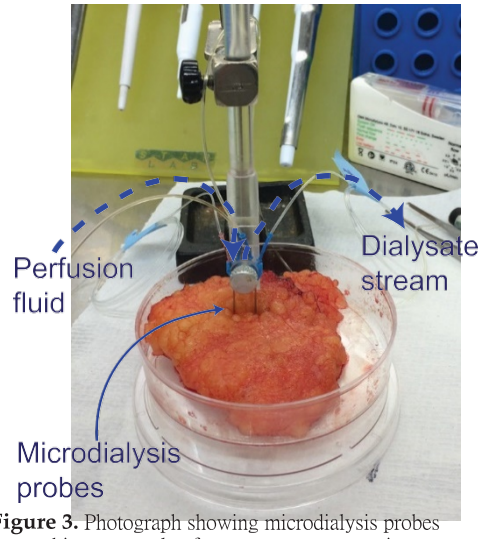

Figure 3. Photograph showing microdialysis probes inserted into a sample of cancerous omentum tissue.

of this concept is shown in figure 4. In addition to detecting diffusion of the drug into the healthy tissue, the second microdialysis probe could interrogate the tissue in real time, giving crucial feedback of any effect on the tissue caused by the drug. This approach would allow individualized chemotherapy treatment and would reduce the severe side effects caused by systemic administration of such drugs.

Liquid biopsy

Despite maximal effort treatment strategies, more than 80% of patients with advanced disease will relapse and die. No valid predictors of relapse have been identified so far, nor do we have a valid way to monitor and diagnose early relapsed disease in clinical practice. Currently CA125 is the only accepted circulating marker for follow-up of ovarian cancer and CA125 levels are monitored during the course of the disease in women undergoing treatment and during follow-up. Nevertheless, this approach has failed to significantly affect survival [37]. There is a clear unmet need for new specific and sensitive biomarkers in ovarian cancer to be surrogates of outcome, prognosis, treatment response, and recurrence. The last decade has seen the emergence of detection of circulating tumour cells (CTCs) and cell-free circulating tumour DNA (ctDNA) in blood as potential non-invasive diagnostic and surveillance strategies for many cancers including ovarian cancer, termed 'liquid biopsies'. A significant advantage of screening CTCs or ctDNA, which can represent the entire tumour genome shed into the circulation, is the reduction in sampling bias compared to invasive single-site tissue biopsies as they may not accurately represent the tumour genome due to the vast heterogeneity that exists in ovarian cancer.

Circulating tumour cells (CTCs)

CTCs were first detected in the blood of a patient with metastatic cancer over a century ago by Ashworth and since then have been identified in the distant metastasis of many different types of solid epithelial cancers [38]. In many tumour types, e.g. prostate [39], breast [40], colorectal [41], and lung [42], the number of CTCs at a given time in circulation has correlated with poor prognosisandincreaseddiseaseburden, demonstrating a potential role as predictor of treatment response and disease progression. Different methodologies are in use to isolate CTCs from peripheral blood, for example size based filtration methods [43], immuno-magnetic antigen (eg EpCAM) dependent methods [44, 45], avidity to type I collagen matrix [46], microfluidics systems to detect cytokeratin positive or negative CTCs [47], negative selection of CTCs via CD45 selection [48], density gradient centrifugation [49, 50] and flow cytometry [51]. The FDA have approved the CTC isolation system CellSearch™ for isolation of CTCs in whole blood [52]. However, the isolation of CTCs is beset by problems. In comparison to other malignancies, very low numbers of CTCs exist in the circulation of patients with ovarian cancer. Some approaches used to isolate CTCs may miss cells in circulation depending on the selection method employed. For example, methods using selection via the tumour marker EpCAM (epithelial cell adhesion molecule/CD326) will miss CTCs which may have lost EpCAM expression via epithelial-mesenchymal transition (EMT). Therefore, it is more prudent to use an approach that does not require separation via epithelial cell markers to ensure collection of allsub- populations of CTCs. Co-isolation of peripheral blood mononuclear cells is also a common problem in isolating CTCs, methodologies employing density- gradient centrifugation or some microfluidics systems may have carry over of leucocytes in their preparations. Downstream PCR profiling of gene expression panels from such populations could be contaminated with leucocyte profiles [53, 54], therefore careful designing of assays to exclude leucocyte contamination is required. Conflicting evidence examining the predictive value of CTCs in ovarian cancer exists; Liu *et al* concluded that CTCs isolated using the CellSearch™ system did not significantly correlate with patient outcome or clinical characteristics [45], however a recent meta-analysis from Zhou *et al* using data from eleven publications showed that CTC status was associated with OS and PFS in ovarian cancer, with subgroup analysis showing that CTC status for OS was significant in the 'RT-PCR' subgrouping and not in the 'CellSearch' subgrouping [55]. Nonetheless, significant progress and standardization of methodologies is essential to improve the isolation and profiling of CTCs, to develop this type of 'liquid biopsy' for the real-time non- invasive monitoring of ovarian cancer.

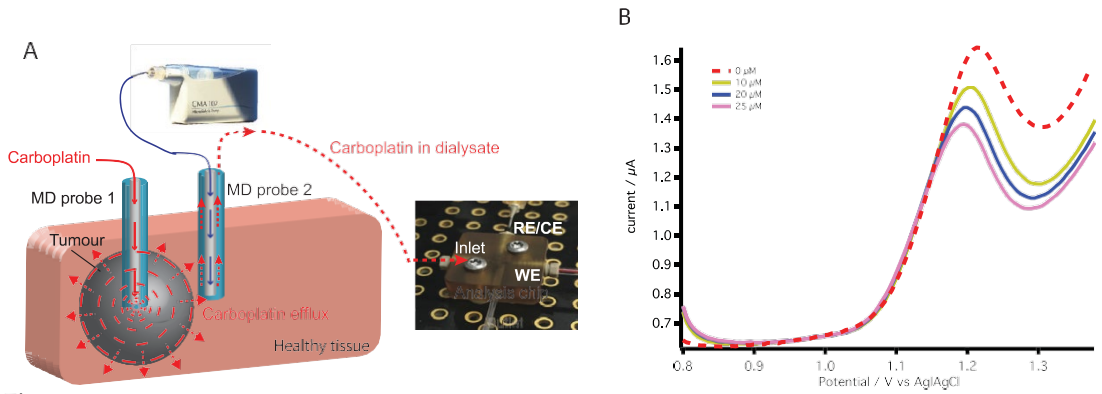

Figure 4. (A) Schematic of online chemotherapy drug delivery and detection system. (B) Proof-of-concept differential pulse voltammetry data showing detection of varying concentrations of carboplatin collected by a microdialysis probe and analysed in real time using a microfluidic sensing system. Reproduced with permission from [36]. CC BY 3.0.

Circulating tumour DNA

Circulating tumour DNA released into the circulation is thought to be derived from necrotic or apoptotic cancer cells [56]. In contrast to CTC isolation, ease of processing from plasma and accessibility to samples makes ctDNA an attractive system to develop for monitoring of cancer burden in patients undergoing therapy and in follow-up, and tracking of clonal evolution of tumours to determine development of resistance to therapy. As with CTC isolation, contamination from leucocyte DNA is possible, therefore plasma collection techniques should be stringent and downstream assays designed to exclude leucocyte DNA cross-over. Sequencing of plasma ctDNA via whole genome, exome or targeted deep sequencing in several studies have shown the feasibility of disease monitoring in ovarian cancer using this approach [57–60]. Exome sequencing of samples collected at different time points from patients tracked over 1–2 years showed changes in copy number and gene specific mutations between samples, and the mutations observed in plasma before and after each treatment course showed differences in their mutation profiles relative to disease progression and drug resistance [61]. In particular, Pereira *et al*, examined ctDNA levels in patients with ovarian cancer and correlated findings with CT scanning and CA125 levels. In a small number of patients, ctDNA detected the presence of cancer when CT scans were negative, and ctDNA was detected an average of 7 months preceding positive CT scans for recurrence [62]. Furthermore, undetectable levels of ctDNA at 6 months following initial treatment was associated with improved PFS and OS [62]. In a recent study, Harris *et al* recently showed that quantification of somatic chromosomal rearrangements in ctDNA from ovarian cancer patients was possible, and was representative of disease burden in patients [63]. In addition, profiling of abnormal plasma DNA in early ovarian cancer using a well characterized non-invasive prenatal testing platform, showed the potential of using this type of high throughput sequencing platform to screen for early HGSOC, the most prevalent type of EOC [64].

Aberrant DNA methylation has been identified in ovarian cancer, with some candidate genes and path- ways showing clinical utility as potential biomarkers [65–67]. Tumour-specific gene methylation has been identified in plasma DNA from patients with ovarian cancer, suggesting a potential diagnostic or prognostic role for screening for epigenetic biomarkers in plasma [68, 69]. Recently, Flanagan *et al* identified that screen- ing for DNA methylation in blood following chemo- therapy could provide a non-invasive method to screen patients' responses to treatment as DNA methylation profiles identified at relapse following chemotherapy correlated with patient survival [70].

Whilect DNA is significantly less problematic to iso- late from blood samples than CTCs, more functional information could be extracted from intact CTCs. Full molecular profiling at the DNA, RNA and protein level is achievable, however standardization of CTC isola- tion methodology is required. Furthermore, the advent of single cell sequencing has increased the capacity to capture the heterogeneity of the CTC population, and inform on sub-clonal variation. However, the signifi- cant ease of sampling of ctDNA and rapid advances in sequencing technologies and epigenetic profiling of ctDNA suggest it may have a more robust clinical utility than CTCs and therefore have a quicker integra- tion into clinical use. Advances in the development and validation of CTCs and ctDNA profiles as biomarkers to inform on patient outcome, allow for personaliza- tion of treatment plans according to the patients' own tumour profile, treatment surveillance, and patient stratification in clinical trials is paramount. The avail- ability of non-invasive 'liquid biopsies' should become commonplace in the future, with serial monitoring of patients accurately informing clinicians on patient pro- gress and responses to therapy.

Palliative treatment of relapsing ascites

Malignant ascites in relapsed ovarian cancer is a therapeutic dilemma. Paracentesis, which involves inserting a large-bore needle into the abdomen to drain 5–10 l of accumulated ascites, is the most

common procedure for the treatment of ascites. However, paracentesis has to be repeated frequently, depending on the tumour burden, however it does not prevent the re-accumulation of ascites. This has serious implications on the patients' quality of life. The Sequana-Medical alfapump-System (AP) is a remotely controlled device connecting the patients' peritoneal cavity to their urinary bladder. Histopathological analysis of the urine has revealed rich malignant cell content that can be used to create FFPE-cell-blocks for molecular-pathological profiling with sequential Caris-Target-Now-analysis and full exome-sequencing. This innovative approach addresses an area of unmet need for the control of malignant ascites and provides a non-invasive method of collecting tumour tissue for continuous molecular tumour characterization during treatment for relapse [71].

Conclusions

There has never been a more fruitful time for surgical advances than now, with implementation of highly modern and revolutionary bioengineering technology into daily surgical techniques to optimize overall surgical outcome and reduce morbidity. Especially in ovarian cancer surgery where the value of radicality is currently being challenged due to a combination of lack of robust prospective evidence and feared high surgical morbidity and mortality, new techniques that rely on better understanding of the tumour biology and overall profiling of the tumour are required to optimize outcomes and minimize morbidity. From bench to bedside this is a prime time for many of those techniques to be validated and established within randomized clinical trials and become an integral part of the surgical management of this challenging disease.

Acknowledgments

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