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EDITORIAL

Translational Therapeutics

New insights into the unique nature of colorectal cancer peritoneal metastases—rethinking HIPEC

Colorectal peritoneal metastases (CRPM) can be resistant to the chemotherapy agent (oxaliplatin) most employed, up until recently, as hyperthermic intraperitoneal chemotherapy (HIPEC). Glutathione-mediated inactivation of oxaliplatin can be substantially reduced by genomic deletion of the gene or pharmacological inhibition of glutamate-cysteine ligase in CRPM tumouroids. These discoveries may rekindle the enthusiasm for HIPEC in concert with cytoreductive surgery, which has been employed to manage patients with this once-nihilistic form of stage-IV disease.

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Colorectal tumour metastasis (mCRC) is the most lethal form of cancer. However, not all metastases are equal as there is the matter of where the metastases are located, their organ-specific volume and whether they have arisen under active systemic therapy or not. These considerations are core to improving outcomes of patients with mCRC and go to the important discoveries reported in this issue of BJC by Laoukili et al. [1].

The metastatic process by which primary CRC disseminates is mostly thought of as a haematogenous or lymphatic journey to the liver and/or lungs. An underappreciated third site is to the abdominal peritoneum, which may be in synchrony with the other two sites. With the advances in hepatobiliary and thoracic surgery, intervention with curative intent to remove liver or lung metastases is routinely entertained and the reported benefits of overall survival justify this modern dogma [2, 3]. The peritoneal disease presents a greater challenge in terms of early detection and clinical management. Relatively morbid, maximally invasive operations, involving the removal of multiple abdominal organs and the peritoneum itself (cytoreduction) followed by direct exposure to chemotherapy agents, in the form of hyperthermic intraperitoneal chemotherapy (HIPEC), are a well-recognised option. Alternatively, where the peritoneal disease is not deemed resectable or the burden is too high to justify resection, another form of intraperitoneal chemotherapy, PIPAC (pressurised intraperitoneal-aerosolised chemotherapy), is being actively pursued as a palliative option [4].

Analysis of CRC gene expression from multiple sources has led to the concept of consensus molecular signatures, CMS1–4 [5], with CRPM predominantly CMS4-like. A number of groups agree on this classification of CRPM and here Laoukili et al. have revealed that the primary cancers from whence the metastases arose are more commonly CMS2- and 3-like. Added to this are the observations that tumouroids derived from CMS4 CRPM show CMS2- and 3-like signatures when allowed to grow out in an extracellular matrix, despite sharing the same mutation and importantly STR fingerprints [6]. Thus, it appears that the gene expression of CRPM is driven by the tumour microenvironment [7].

A characteristic of CMS4 tumours is chemotherapy resistance and relatively poor patient survival, which is a particularly serious issue in CRPM, given the lack of new systemic agents for frontline treatment. 5-fluorouracil (5-FU) has served as a backbone agent either in combination with oxaliplatin or irinotecan and, indeed, in combination together. In addition, the majority of peritonectomy centres rely upon either oxaliplatin or an even older chemotherapy agent—mitomycin C as their HIPEC drug of choice. Huge controversies range following the delayed publication of the PRODIGE-7 clinical trial, where the addition of oxaliplatin-based HIPEC to expertly performed cytoreductive surgery failed to translate into any survival benefit [8]. There is much to unpack about the use of either agent in the context of intraperitoneal therapy, but what is clear is that better chemotherapy agents are needed or perhaps that strategies that improve the action of the current agents are urgently required.

Patient-derived organoids (PDO) or tumouroids are providing new promise as a rapidly generated and tumour-specific platform for probing drug sensitivities of tumours in a clinically tractable window of time [9]. Peritoneal tumouroids are particularly useful in this regard, because in most cases, biopsies can be retrieved laparoscopically at the time of patient evaluation or while on treatment. Of note is that multiple sites that are typically a feature of CRPM can be sampled and assessed individually, established with a 70% or better success rate, and drug sensitivity can be determined in less than a month.

Laoukili et al. tested such tumouroids for sensitivity to oxaliplatin being mindful that, clinically, the exposure to oxaliplatin is short (not more than an hour) and heated like it would be if used in a HIPEC procedure. Based on the stromal-rich CMS4-like signature, they connected the knowledge that this signature is driven predominantly by cancer-associated fibroblasts or CAFs. By the time, the tumouroids are established, CAFs are left behind. Nevertheless, a common additive to the complex cocktail of organoid cultures is N-acetylcysteine (NAC,) which is a precursor for glutathione provided in vivo by CAFs. Importantly, the addition of NAC does not appear to be enough to maintain/establish a CMS4-like signature in tumouroids [6], but it can be omitted.

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When omitted, 72 h of exposure of tumouroids to oxaliplatin increases drug sensitivity markedly. One-hour oxaliplatin exposure, which recapitulates the upper end of HIPEC exposure, revealed modest tumouroid killing and omission of NAC had little effect. This was a valuable insight. Another important observation the authors made, that is not explored enough elsewhere, is to ask if a treated tumouroid can initiate regrowth over an extended time (3 weeks). The ability to "self-renew" is a key hallmark of cancer-threatening tumour recurrence, which happens often in patients following HIPEC. In summary, if NAC is omitted and the exposure of tumouroids is sufficiently long (e.g., 72 h), oxaliplatin can kill peritoneal tumour cells very effectively and regrowth is substantially lost.

The final part of this study elevates its impact with implications for clinical translation. By connecting the dots, implicating glutathione synthesis and oxaliplatin inactivation by glutathione (GSH), they discovered elevated glutamate-cysteine ligase (GCLC) protein expression in peritoneal tumoroids. CRISPR–Cas9-mediated *GCLC* knockout closed the reasoning-circle-sensitising tumouroids to oxaliplatin but not irinotecan or 5-FU. The direct evaluation of platinum adducts on DNA increased by *GCLC* KO and blocked by GSH was a nice mechanistic conformation of the mode of action. Two gaps remained. Is there a clinically viable pharmacological intervention to affect GCLC and one that would allow oxaliplatin to effectively kill metastases within the 1-h window used by HIPEC? To this end, buthionine sulfoximine (BSO) is a GCLC inhibitor or with APR-246, which reduces GSH [10]. BSO, at least, allows oxaliplatin to kill tumouroids effectively within the 1-h treatment period and, importantly, impedes long-term regrowth.

In summary, this study by Laoukili et al. restores some hope that there might be a place for oxaliplatin as a HIPEC drug option, if indeed it is allowed to generate DNA adducts in CRPM. It is conceivable that 1 h at 42 °C may be sufficient, if agents like BSO or APR-246 are co-delivered, whereas previously, this was probably unlikely with oxaliplatin alone. Although not tested, mitomycin C, which is instilled intraperitoneally for slightly longer times, is argued to be similarly affected by GSH-mediated inhibition. The chemistry of inactivation of both cross-linking agents is claimed to be similar and this will need to be confirmed. This is a very pressing question as the switch away from oxaliplatin back to mitomycin C by the oncological community may be premature if oxaliplatin is permitted to do its work. Other questions now emerge. APR-246, for instance, is subject to drug-transporter requirements afforded by solute carrier SLC7A11 [10]. Perhaps, such potential biomarkers will need to be explored in CRPM as well. There are always new research questions.

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DATA AVAILABILITY
Not applicable.

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