Title: Clinical development of PD-1/PD-L1 immunotherapy for gastrointestinal cancers: facts and hopes

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CONFLICT OF INTEREST

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ABSTRACT

Gastrointestinal (GI) cancers are among the most deadly malignancies. Whereas serial incremental survival benefits have been made with cytotoxic chemotherapy with metastatic disease, a plateau of achievement has been reached. Applying modern integrative genomic technology, distinct molecular subgroups have been identified in GI cancers. This not only highlighted the heterogeneity in tumours of each primary anatomical site, it also identified novel therapeutic targets in distinct molecular subgroups and might improve the yield of clinical success. Molecular characteristics of tumours and their interaction with tumour microenvironment would further impact on development of combination therapy, including immunotherapy. Currently immune checkpoint blockade attracts the most intense research and the successful integration of these novel agents in GI cancers in the treatment paradigm requires an in-depth understanding of the diverse immune environment of these cancers.

INTRODUCTION

Cancers of gastrointestinal (GI) tract are among the most deadly malignancies with a high mortality to incidence ratio. Oesophago-gastric (OG), pancreatic, liver and colorectal (CRC) cancers account for more than 2,894,000 deaths per annum (1). Whereas serial incremental survival benefits have been made with cytotoxic chemotherapy with metastatic OG and colorectal cancers, a plateau of achievement has been reached. Applying modern integrative genomic technology, distinct molecular subgroups have been identified in GI cancers. This not only highlighted the heterogeneity in tumours of each primary anatomical site, it also identified novel therapeutic targets in distinct molecular subgroups and might improve the yield of clinical success. Molecular characteristics of tumours and their interaction with tumour microenvironment would further impact on development of combination therapy, including immunotherapy.

Currently immune checkpoint blockade especially targeting Programmed Death -1 (PD-1) and Programmed Death Ligand (PD-L)1 attracts the most intense research. PD-1 is a cell surface co-inhibitory receptor expressing in T cells, B cells, monocytes and natural killer (NK) cells. It has two known ligands – PD-L1 and PD-L2. PD-L1 is up-regulated by tumour cells and by cells in the tumour microenvironment. PD-1 interaction with its ligands inhibits T-cell receptor signalling and down-regulates T-cells responses. Inhibition of PD-L1 could restore T cell activity against tumour cells, thereby preventing cancer metastasis and reducing tumour volume (2). This review focuses on the current and future approach of immunotherapy and its interface with the recent genomic data from GI cancers.

OESOPHAGO-GASTRIC CANCER

There have been several large scale research efforts to ascertain molecular subgrouping for gastric cancer. Notably the Cancer Genome Atlas (TCGA) identified four groups – Epstein Barr Virus (EBV) infected (9%), Microsatellite Instability (MSI 22%). Genomically Stability (GS 20%) and Chromosomal Instability (CIN 50%) (3). The Asian Cancer Research Group (ACRG) also described 4 subgroups – MSI (22.7%), Microsatellite Stable (MSS)/ epithelial-to-mesenchymal transition (EMT 15.3%), MSS/TP53 positive (26.3%) and MSS/TP53 negative (35.7%) (4). However of note the four subgroups described by the TCGA did not carry any prognostic effect, although this might be partly due to the tumour samples deriving from operable OG cancers with limited follow-up (3). Furthermore, several molecular aberrations overlapped between different subgroups and thus these might not be completely distinct subgroups. For example *PIK3CA* mutations were frequently observed in the EBV subgroup, but were also found, albeit less frequently, in the MSI, GS and CIN subgroups. In contrast the four subgroups identified by the ACRG did have statistically significant survival differences (4). This prognostic difference between TCGA and ACRG was not necessarily related to the limited follow-up of the TCGA. In addition, the semi-supervised analysis used by the ACRG with the incorporation of clinical characteristics might have contributed to this difference (3, 4). Within the EBV-infected and MSI gastric cancer described in the TCGA, there were significantly higher expression of PD-L1 in both the tumour cells and immune cells compared to other subgroups (5, 6). Furthermore interferon-γ gene set enrichment was also more frequently seen in the EBV-infected and MSI subgroups, although there was no association between interferon-γ signature and total number of mutations (5). These subgroups might be particularly sensitive to PD-L1 blockade and of enhanced

relevance especially MSI gastric tumours might have a negative prognostic impact when treated with cytotoxic chemotherapy (7).

The initial enthusiasm in targeting PD-L1 in gastric adenocarcinoma came from the results of pembrolizumab in PD-L1 positive gastric cancer in the KEYNOTE-012 study (8). In this study, patients from both Asian and non-Asian countries were enrolled. Forty per cent of screened population were found to be PD-L1 positive in a relatively heavily pre-treated patient population. An objective response rate (ORR) of 22% on central review and durable responses were seen with median duration of responses of 40 weeks. Six-month progression free survival (PFS) rate was 26% and impressively the median overall survival (OS) was 11.4 months with 12-month OS rate of 42%. Based on these, several randomised controlled trials (RCTs) have been/are being performed. Table 1 shows selected on-going randomised studies evaluating PD-(L)1 antibodies in GI cancers. In the second line KETNOTE-061 RCT, patients were not initially pre-selected for tumour PD-L1 expression, but PD-L1 positive patients were enriched at a latter part of the study. In the first line KEYNOTE-062 study, only patients with PD-L1 positive OG cancer are being recruited as this 3-arm RCT has a pembrolizumab alone treatment arm without any cytotoxic chemotherapy and data from KEYNOTE-012 were on PD-L1 positive gastric cancer alone.

Nivolumab has also been evaluated in a number of studies in gastric cancer. In the CHECKMATE 032 study, both nivolumab monotherapy and the combination of nivolumab plus ipilimumab were tested in gastric cancer patients. The combined PD-L1 and CTLA-4 targeting were first found to be valuable in malignant melanoma (9),

but more recently in other tumours such as small cell lung cancer (10). CHECKMATE 032 gastric cohort recruited 160 gastric cancer patients who were allocated non-randomly to nivolumab (3mg/kg) monotherapy (n=59) and two dose schedules of nivolumab plus ipilimumab – nivolumab 1mg/kg and ipilimumab 3mg/kg (nivo 1 ipi 3; n=49) or nivolumab 3mg/kg + ipilimumab 1mg/kg (nivo 3 ipi 1; n=52) (11). Similar to KEYNOTE-012, a heavily pre-treated patient population was recruited with 79% of patients had had ≥2 prior therapy. However unlike KETNOTE-012, patients were enrolled irrespective of PD-L1 expression status and all patients were of Western population. The ORR was 14% (nivo alone), 26% (nivo 1, ipi 3) and 10% (nivo 3, ipi1). The median duration of response was 7.1 months, 5.6 months and not reached respectively. Six-month PFS was 18%, 24% and 9% and 12 month OS was 36%, 34% and not available respectively. There was some correlation between ORR and PD-L1 expression for nivo alone, but less so with the combination of nivo and ipi similar to the observation in malignant melanoma (9, 11).

Most recently a phase III placebo-controlled RCT was reported for nivolumab in third or subsequent line therapy. The ONO12 (ATTRACTION-2) study only recruited patients in Korea, Japan and China and thus consisted entirely of Asian population (12). In this large study, 493 patients were randomised in a 2:1 fashion to nivolumab or placebo. Nivolumab resulted in statistically superior OS (hazard ratio [HR]: 0.63; 95% confidence interval [CI]: 0.50-0.78; p<0.0001), PFS (HR: 0.60; 95%CI: 0.49-0.75; p<0.0001) and ORR. Twelve month OS rates were 26.6% and 10.9% and ORR was 11.2% vs 0% for nivolumab and placebo respectively. Whereas the ONO-12 and CHECKMATE 032 showed similar efficacy for nivo alone in both Asian and Western populations, it has been previously shown that the Asian and non-Asian gastric cancer

might exhibit distinct gene signatures related to inflammation and immunity (13). In particular, immune T cell expression signatures were enriched in non-Asian gastric cancers including both CD28 and CTLA-4 signalling with supportive immunohistochemistry data showing T-cell markers (CD3, CD45R0 and CD8) significantly enriched in Caucasian compared with Asian GC. The exception was the immunosuppressive T-regulatory cell marker FOXP3 which was significantly enriched in the Asian population. These immune-related differences were however unrelated to EBV infection and MMR status.

Avelumab, an anti-PD-L1 antibody, had been evaluated in a phase IB expanded cohort JAVELIN study in two different settings – maintenance post first line therapy and second line treatment (14). In the second line setting, ORR was similar to nivolumab and pembrolizumab. Maintenance setting has so far not been explored by other PD-1 antibodies and forms the current registration strategy for avelumab in gastric cancer (Table 1).

When one interrogated the integrated molecular description of gastric cancer in the TCGA, both JAK2/PD-L1/2 and VEGF A were altered in the CIN subgroup. Targeting angiogenesis is now an established treatment options in gastric cancer (15, 16). Vascular Endothelial Growth Factor Receptor (VEGFR) 2 pathway activation by VEGF-A might suppress antitumour T cell activation by i) blocking the maturation of dendritic cells disrupting tumour antigen presentation; ii) inducing the expression of PD-L1 on dendritic cells; iii) enhancing regulatory T-cell which could inactivate antitumour immune cells (17, 18). In addition, preclinical evidence has shown low vascular normalizing doses of anti-angiogenics such as DC101 (murine parent

antibody to ramucirumab targeting VEGFR2) reprogrammed the tumour microenvironment from immunosuppressive to immunosupportive and potentiated immunotherapy (19). In contrast high dose DC101 might prune tumour blood vessels and promote immunosuppressive tumour microenvironment. Furthermore, there were further data suggesting synergistic inhibitory effect of DC101 with anti-PD1 antibody in a colon adenocarcinoma murine model (20).

With this background, a phase 1 study was initiated combining pembrolizumab and ramucirumab in PD-L1 unselected multi-tumour patient cohorts. Second/third line gastric cancer cohorts were examined in two different dose schedule (low dose or high dose ramucirumab plus fixed dose pembrolizumab) (21). A further first line chemonaïve cohort was also being explored. The safety profile of ramucirumab combined with pembrolizumab was favourable allowing administration of each drug at full dose. Some anti-tumour activity was observed in previously treated gastric adenocarcinoma but data were immature for survival endpoints (21). No results are currently available for the chemonaïve cohort. Similar to targeting angiogenesis, another combination strategy of targeting the tumour microenvironment with immune-oncology (IO) compounds would be against matrix metalloproteinase (MMP)-9 and PD-L1. A randomised phase 2 study of nivolumab with or without andecaliximab (GS-5745) is on-going (Table 1).

There are a number of adaptive designed phase II studies either recruiting or being planned to combine different IO agents in gastric cancer. For example FRACTION (A phase 2, Fast Real-time Assessment of Combination Therapies in Immuno-Oncology study in patients with advanced gastric cancer) is currently randomising

nivo + ipi with nivo + BMS-986016 (anti-LAG3 antibody), with further combination IO compounds to be added in future (Table 1).

TCGA also recently reported the genomic characterisation of oesophageal carcinoma (22). As one would expect, squamous cell carcinoma (SCC) and adenocarcinoma were molecularly distinct. Oesophageal adenocarcinoma strongly resembled the CIN variant of gastric adenocarcinoma, although DNA hypermethylation occurred disproportionately in oesophageal adenocarcinoma. No oesophageal adenocarcinomas were positive for MSI or EBV. In contrast SCC revealed frequent alterations in cell cycle regulators with inactivation of *CDKN2A* and amplification of *CCND1*. Furthermore EGFR amplification or mutation was seen in 19% and alterations of PIK3CA, PTEN or PIK3R1 in 24% of tumours. TCGA divided oesophageal SCC into three molecular subtypes: ESCC1 characterised by alteration in NRF2 pathway and resembled more close to lung and Head & Neck SCC; ESCC2 showed higher rate of mutation of *NOTCH1* or *ZNF750* and greater leucocyte infiltration; ESCC3 sustained alterations predicted to active the PI3K pathway. With the success of nivolumab in SCC Head and Neck (23) and Lung (24), ESCC1 might be a subgroup more susceptible to PD-1 targeting.

The first study of targeting PD-1 in SCC oesophagus was recently published (25). This was conducted entirely in Japan with 65 patients enrolled and it was unselected for tumour PD-L1 positivity. An ORR of 17% on central review and median OS of 10.8 months were observed. Interestingly immune-related ORR was 25%.

COLORECTAL CANCER

Similar to OG cancer, there has been several large scale efforts to define molecular subgroups in CRC. Indeed a consensus molecular subgroup (CMS) has been proposed (26). CMS1 (MSI Immune), CMS 2 (Canonical), CMS 3 (Metabolic) and CMS 4 (Mesenchymal) had different molecular characterisation. Of particular interest CMS 1 had MSI and thus hypermutation with immune infiltrate activation. This subgroup had worse survival after relapse. For CMS 4 mesenchymal, there was stromal infiltration, TGF-β activation and angiogenesis. This subgroup had worse relapse-free and overall survival.

The immune landscape of these CMS had also been explored (27). CMS 1 and 4 had high expression of lymphoid as well as myeloid cell-specific genes, thus exhibiting a strong immune and inflammatory contexture. However, the poor prognostic CMS 4 differed from CMS1 with higher expression of endothelial cell and fibroblast genes. In addition, functional relevant immune genes were also up-regulated in CMS 1 and CMS 4. CMS1 exhibited a high expression of genes coding for T-cell-attracting chemokines or involved in formation of tumour adjacent tertiary lymphoid structures, all associated with good prognosis in CRC. In contrast CM4 exhibited high expression of myeloid chemokine, angiogenic factors and immune-suppressive molecules (27). CMS2 and CMS3 might potentially be “immune deserts” consisting up to 50% of CRC cases whereas CMS1 and CMS 4 resembled more of “immune paradise” although CMS 4 also had inflammatory and angiogenic components (Figure 1).

In the early development of anti-PD (L)1 antibodies, only marginal if any benefit was seen in metastatic CRC (mCRC). Much more encouraging efficacy was seen in a small subgroup of patients with mismatch repair (MMR) deficient mCRC. This subgroup only constituted ~4-5% of all patients with mCRC. ORR of 62% was reported in 13 patients with MMR-deficient CRC using pembrolizumab whereas no response was observed in 25 patients with MMR-proficient CRC (28). In this study, 85% of patients with MMR deficiency were of Lynch Syndrome families. Preliminary PFS and OS were all superior in the MMR deficient compared to MMR proficient patients (p=0.001 and p=0.03 respectively) when treated with pembrolizumab (28). In an updated analysis, a total of 53 patients (28 MMR deficient and 25 MMR proficient) were treated. ORR was 50% for MMR deficient CRC and 0% for MMR proficient CRC, respectively. For MMR deficient CRC, the PFS rates was 61% at 24 months and the OS rate was 66% at 24 months (29). The number of somatic mutations was significantly higher in the MMR-deficient tumours compared to MMR proficient and this correlated with objective response (28). Mutation rate and neoantigen load might contribute to sensitivity to anti-PD-1 antibodies (30, 31), although this might not be an universal phenomenon in GI cancers.

A further study was recently reported evaluating nivolumab ± ipilimumab for MMR deficient mCRC (32, 33). Larger number of patients were recruited in this study (n=74) and ORR of 20% was observed for nivolumab monotherapy. Twelve-month PFS and OS rates were 48% and 74% respectively. The proportion of Lynch family in this study was 31%. Responses were seen regardless of tumour PD-L1 expression, abundance of PD-L1 expressing tumour-associated immune cells, B raf mutation or Lynch syndrome. In addition there was improvement of QoL observed after

nivolumab approaching the population norm (32). The combination of nivo + ipi resulted in ORR of 33.3%. Six month PFS and OS rates were 66.6% and 85% respectively with the combination (33). Similar to pembrolizumab study, only one response was seen out of 20 patients with MMR proficient tumours.

In mCRC, the challenge remains how to convert the MSS tumours and "immune desert" like CMS2 and CMS3 to be more responsive to immunotherapy. There was preclinical evidence to suggest MEK inhibition alone could result in intratumoral CD8+ effector T-cell accumulation and MHC 1 up-regulation. This synergised with anti-PD1 agent to promote durable tumour regression (34). With this rationale, a phase 1 study of cobimetinib (MEK inhibitor) with atezolizumab (PD-L1 antibody) was conducted with an expanded cohort in mCRC (n=23). ORR of 17% was observed in all mCRC patients and 20% in *KRAS* mutant CRC cohort (n=20) (35). This contrasted with almost 0% response seen in MSS patients treated with PD-1 antibodies. Based on this, a phase III trial (COTEZO) has completed recruitment for mCRC patients at third or subsequent line treatment randomising to regorafenib (control), atezolizumab or cobimetinib plus atezolizumab (Table 1).

Aside from PD1 blockade, there are other immunotherapy strategies being actively pursued in mCRC. Bispecific T cell engager (BiTE) are molecules that recruit and engage T cells through simultaneous binding to the CD3ε subunit of the T cell receptor complex and a tumour surface antigen, which results in T cell cross linking (36). One FDA-approved BiTE is blintumumab, a CD19/CD3 BiTE used for acute lymphoblastic leukaemia. For mCRC and GI cancers generally, CEA would be a suitable tumour surface antigen. MEDI-565/AMG 211 was a CEA and CD3

bispecific single-chain antibody and it was found in preclinical model that it mediated T cell directed killing of CEA positive cells and this activity was independent of the mutational status of cancer cell lines such as *KRAS/BRAF/PI3KCA* mutation, loss-of-function mutation in TP 53 or PTEN loss (37). This preclinical activity was also seen in patients' CEA+ CRC specimens (38). A phase 1 study was initiated for MEDI-565 for GI adenocarcinoma. Thirty-nine patients were recruited with the majority having mCRC. The dose limiting toxicities seen were hypoxia and cytokine release syndrome (39). Unfortunately no objective response was seen and it was unclear how many patients had CEA-positive tumours.

Another new CEA-T cell bispecific antibody (TCB) has been developed (RG7802) and this CEA TCB had a bivalent binding to CEA and monovalent binding to CD3ε subunit of T cell receptor (36, 40). Preclinically CEA TCB was found to mediate efficient T cell dependent tumour cell lysis by inducing stable crosslinking of multiple T cells to individual tumour cells. It demonstrated efficacy in non-inflamed and poorly T-cell infiltrated tumours. It increased T cell infiltration in tumours converting non-inflamed, PD-L1 negative tumours to highly inflamed PD-L1 positive tumours leading to a more inflamed tumour microenvironment (36, 40) which also paved the way to future combination of CEA TCB and PD-(L) 1 antibodies. Cergutuzumab amunaleukin (RO6895882) is another strategy of immunocytokine which consists of a variant of interleukin 2 that targets CEA. A combination study of RO6895882 and atezolizumab is being performed in various CEA-expressing solid tumours including colorectal and pancreatic cancers.

Another approach that has been evaluated in mCRC was activating innate immune response with toll like receptor (TLR) agonists (41). TLRs are key components of the innate immune system and are essential for the recognition of pathogen-associated molecular pattern (PAMP) and/or damage-associated molecular pattern (DAMP) molecules. The release of DAMP resulted from non-infectious inflammatory response. Whereas host inflammatory cells would attempt to destroy malignant cells, if this acute inflammatory response was insufficient to fully destroy the developing tumour, a dysregulation of the immune system could occur resulting in a chronic inflammatory response typified by production of large numbers of certain innate immune cells ultimately promoting the growth and progression of cancer (42, 43). Currently 10 human TLRs have been identified. TLR-9 is located in the plasmacytoid dendritic cells, but also expressed on majority of innate and adaptive (CD4+, CD8+, NK T and γδ T) effector cells and in B cells. During tumour cell death, mtDNA and mitochondrial formyl peptides are released, which may act as DAMP and potentially result in the dysregulation of TLR-9.

The first generation TLR-9 agonist developed was a CpG oligodeoxynucleotides (CpG-ODN) called PF3512676. Unfortunately when added to standard chemotherapy in non-small cell lung cancer, no survival benefit was seen (44, 45). The second generation TLR-9 agonist also halted development due to toxicity and lack of efficacy. The next generation TLR-9 agonist, lefitolimod (MGN 1703) underwent further structural changes which might improve efficacy and safety. Lefitolimod has been found to be much more potent than CpG-ODN with evidence of immune activation in heavily pre-treated patients with solid tumours and mCRC in particular. Both innate and adaptive immune responses were seen *in vivo* (41). Lefitolimod was

tested in a small randomised placebo controlled trial as maintenance therapy. Patients who had completed first line therapy with oxaliplatin or irinotecan/ fluoropyrimidine ± bevzcizumab were randomly allocated to lefitolimod or placebo (46). There was a trend towards better PFS with lefitolimod from randomisation and statistically significant better PFS from start of induction therapy. In addition, the greatest benefit of lefitolimod appeared to be in patients with relatively low tumour burden. Therefore IMPALA, the phase III RCT of lefitolimod, is recruiting 540 mCRC patients with at least partial responses to first line therapy as maintenance therapy. The addition of pembrolizumab to TLR-4 agonist is also being evaluated in other tumours such as follicular lymphoma. A phase 1b/2 study is designed to evaluate intratumoural G100 (TLR-4 agonist) plus local radiation and pembrolizumab versus G100 plus local radiation alone in patients with follicular lymphoma (NCT: 02501473).

As prognostication, the Immunoscore has gained much recent attention. Taking into the account of the proportion of cytotoxic and memory T cells as well as their location – tumour centre or invasive margin (47, 48), patients with high Immunoscore had a much more favourable time to recurrence in a recent multinational validation project (49). The challenge is whether Immunoscore could be incorporated into predictive biomarker for checkpoint inhibitors in mCRC.

PANCREATIC CANCER

Integrated analysis of genomic, epigenomic and transcriptomic characteristics in pancreatic ductal adenocarcinoma (PDAC) had also identified 4 molecular subtypes: squamous; pancreatic progenitor; aberrantly differentiated endocrine exocrine

(ADEX) and immunogenic (50). Yet checkpoint inhibition in PDAC had been disappointing thus far. The immune environment of PDAC might be particularly hostile. This was characterised by i) sparse intratumoural cytotoxic CD8+ effector T cells; ii) *RAS* oncogene driving inflammatory programme; iii) CD3+ T cell sequestering preferentially at tumour margin and iv) excessive immunosuppressive leucocytes in tumour microenvironement (51). In a recent pooled analysis of TCGA and ICGC (International Cancer Genome Consortium) PDAC data (52), it was found that T cells were present but inactive. Robust tumour infiltrating lymphocytes were present but T-cell activation signature was absent. Contrary to other tumours, high mutation load in PDAC was inversely related with T-cell activity (52).

PD-L1 positive PDAC had less favourable prognosis although expression was sparse (53). In a phase II study of ipilimumab in metastatic pancreatic cancer, no objective response was observed out of 23 patients although 1 patient had immune related response (54). In another study of ipilimumab with or without GVAX, a vaccine based on allogeneic pancreatic tumour cells genetically modified to produce GM-CSF, again no responses was seen although decrease in CA19-9 marker level was observed when GVAX was added (55). Furthermore in a phase 1 study of PD-L1 antibody, there were no responses seen out of 14 patients with PDAC (56). In MMR deficient pancreatic cancer, objective responses have been seen with pembrolizumab but patient number was extremely limited (57).

The largest study evaluating immunotherapy approach in advanced PDAC was the TELOVAC study (58). GV1001, a human telomerase reverse transcriptase catalytic subunit (hTERT) class II peptide vaccine was given either sequentially after

chemotherapy or concomitantly with chemotherapy. Gemcitabine plus capecitabine was given both as control arm and also as chemotherapy with GV1001 in the experimental arms. TELOVAC was probably the largest RCT ever conducted in advanced PDAC with 1,002 patients randomised to these three arms. Unfortunately no survival benefit was seen with this immunotherapeutic approach (58).

The "immune desert" of pancreatic cancer represented a major therapeutic challenge. Interestingly preclinically, similar to mCRC, there was evidence that combining MEK and PD-1 inhibition exhibiting greater inhibitory effect on tumour growth compared to PD1 inhibition or MEK inhibition alone (59). Myeloid cells protected pancreatic tumour cell viability by blocking CD8+ T cell-mediated anti-tumour response. This was achieved by activating PD-1/PD-L1 checkpoint through EGFR/MAPK signalling. Depletion of myeloid cells in the microenvironment arrested tumour growth or induced tumour regression. Increased level of immunosuppressive leucocytes and desmoplastic stroma forming barrier to T-cell infiltration represented critical obstacles to immunotherapy in PDAC. Focal adhesion kinase (FAK) might be important for regulating fibrotic PDAC tumour microenvironment. FAK inhibition induced tumour stabilisation, decreased fibrosis without accelerating tumour progression and decreased immunosuppressive cell population in tumours (60). FAK inhibition (FAKi) improved survival the most with FAKi + Gemcitabine + antiPD1/antiCTLA 4 therapy in mice bearing transplantable *KRAS* mutated pancreatic tumours (60).

BILARY TRACT CARCINOMA

Primary biliary tract cancers (BTC) consists of cholangiocarcinoma – intrahepatic (ICC) and extrahepatic (ECC) as well as gallbladder cancer. They arise from

malignant transformation of biliary epithelium, typically occurring in the setting of chronic inflammation (61). Expression of PD-1 and PD-L1 is up-regulated in ICC tumour tissues (62). Increased expression of PD-L1 was associated with poor differentiation and stage of ICC whereas increased CD8+ T cells in tumours was associated with better tumour differentiation.

In a series of resected ICC, the majority of tumours expressed PD-L1 on tumour cells located either within the tumour front or on tissue-associated macrophages (TAMS) (63). PD-L1 expression was found to be associated with nodal metastasis and larger number of lesions. PD-L1 expression within the tumour front was associated with worse survival, suggesting the PD-1 pathway might be suppressing the host immune response in ICC (63). In another study, all resected ICC expressed PD-1 which was present on tumour-infiltrating lymphocytes but was not detected on ICC cells (64) . Whereas immune infiltrate was present in all tumour analysed, the proportion of CD8+ T cells was significantly higher in the fibrous septa compared to tumour lobules (64). ICC tumours with down-regulation of HLA Class I antigen expression presented with more advanced stage. Therefore this microenvironment might potentially attractive for an PD-(L) 1 antibody therapy.

There is paucity of clinical data in the use of anti-PD-(L)1 antibody in advanced BTC. Pembrolizumab was tested in a multi-cohort phase IB KEYNOTE – 028 (65). Only patients with PD-L1 positive tumours were recruited. Of the screened advanced BTC population, 42% were PD-L1 positive. Twenty-four patients were recruited and ORR of 17% was observed (65). In the aforementioned phase 1 study evaluating pembrolizumab plus ramucirumab in gastric cancer patients, there was a separate

cohort of advanced BTC patients which have completed recruitment, but no results are available yet.

Hepatocellular carcinoma has distinct immune environment which might represent potential “immune paradise” for checkpoint inhibition. However a separate review article will focus on this particular subject.

CONCLUSIONS

There are still much data with immunotherapy in GI cancers not discussed in this review – active immunotherapy with dendritic cell vaccine, viral vector vaccine; passive immunotherapy like chimeric antigen receptor (CAR) T cell therapy and other checkpoint modulators such as LAG3, OXO40, KIR, TIM-3. Combining radiotherapy (RT) with PD-(L) 1 antibodies to enhance anti-tumour T cell response and augment abscopal effect is also being actively pursued in GI cancers. This abscopal effect where a non-irradiated site regresses after RT to the primary tumour might stem from an immune-related mechanism and synergistic effect of RT and PD-(L)1 antibodies hyave been observed in preclinical models. The immune landscape of GI cancer is wide-ranging with immune paradise and immune desert. Integrating information collected from genomic analysis and immune microenvironment would hopefully turn these into immune oasis and provide greater opportunities in immunotherapy for the greater benefits of our patients.

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Table 1 Selected on-going randomised studies evaluating PD-(L)1 antibodies in gastrointestinal cancers

Trial protocol / NCT ID	*Study setting*	*Phase*	*Treatment Arms*	*Planned recruitment number of patients*	*Primary outcome*
Gastric and OGJ cancers					
ONO-4538-38 NCT 03006705	Adjuvant	III	**1)** CAPOX or S-1 + placebo **2)** CAPOX or S1 + nivolumab	700	Relapse free survival
FRACTION NCT 02935634	Advanced/ metastatic ≥2nd line	II adaptive	**1)** Nivolumab + ipilimumab **2)** Nivolumab + BMS-986016 (anti-LAG 3 antibody) **3)** Nivolumab + other IO compounds	910	ORR DOR PFS rate
ONO-4538-37 NCT 02746796	Advanced/ metastatic 1st line	II	**1)** SOX or CAPOX **2)** SOX or CAPOX + nivolumab	268	ORR
CHECKMATE 649 NCT 02872116	Advanced/ metastatic 1st line	III	**1)** CAPOX or FOLFOX **2)** CAPOX + nivolumab **3)** Nivolumab + ipilimumab	1,266	OS
GS-US-296-2013 NCT 02864381	Advanced/ metastatic ≥2nd line	II	**1)** Nivolumab **2)** Nivolumab + GS-5745 (anti-MMP 9 antibody)	120	ORR

Trial protocol / NCT ID	*Study setting*	*Phase*	*Treatment Arms*	*Planned recruitment number of patients*	*Primary outcome*
KEYNOTE 061 NCT 02370498	Advanced/ metastatic 2nd line	III	**1)** Paclitaxel **2)** Pembrolizumab	720	OS/ PFS
KEYNOTE 062 NCT 02494583	Advanced/ metastatic 1st line	III	**1)** Cisplatin + fluoropyrimidine + Placebo **2)** Cisplatin + fluoropyrimidine + pembrolizumab **3)** Pembrolizumab	750	PFS/ OS
KEYNOTE 063 NCT 03019588	Advanced/ metastatic 2nd line	III	**1)** Paclitaxel **2)** Pembrolizumab	360	OS/ PFS
JAVELIN GASTRIC 300 NCT 02625623	Advanced/ metastatic 3rd line	III	**1)** Best supportive care **2)** Avelumab	330	OS
JAVELIN GASTRIC 100 NCT 02625610	Advanced/ metastatic 1st line maintenance	III	**1)** Continuation of 1st line chemotherapy **2)** Avelumab	666	OS
PLATFORM NCT 02678182	Advanced/ metastatic 1st line maintenance	II	**1)** Observation **2)** Capecitabine **3)** Durvalumab	616	PFS

Trial protocol / NCT ID	*Study setting*	*Phase*	*Treatment Arms*	*Planned recruitment number of patients*	*Primary outcome*
Oesophageal cancer					
CHECKMATE 473 ONO-4538-24 NCT 02569242	Advanced/ metastatic ≥2nd line	III	**1)** Docetaxel or paclitaxel **2)** Nivolumab	390	OS
KEYNOTE 181 NCT 02564263	Advanced/ metastatic ≥2nd line	III	**1)** Docetaxel or paclitaxel **2)** pembrolizumab	600	PFS/ OS
CHECKMATE 577 NCT 02743494	Adjuvant Post pre-op CRT Oesophageal and OGJ	III	**1)** Placebo **2)** nivolumab	760	DFS/ OS
NCT 02520453	Adjuvant Post pre-op CRT	II	**1)** Placebo **2)** Durvalumab	84	DFS/ OS
Colorectal cancer					
KEYNOTE 177 NCT 02563002	Advanced/ metastatic MSI-H/ dMMR 1st line	III	**1)** Standard of care chemotherapy (FOLFOX or FOLFIRI ± bevacizumab ± cetuximab) **2)** Pembrolizumab	270	PFS

Trial protocol / NCT ID	*Study setting*	*Phase*	*Treatment Arms*	*Planned recruitment number of patients*	*Primary outcome*
NCI 170057 **NCT 03050814**	Advanced/ metastatic 1st line	II	**1)** FOLFOX + bevacizumab **2)** FOLFOX + bevacizumab + Ad-CEA + avelumab	81	18-month disease progression rate
NCIC CO26 **NCT 02870920**	Advanced/ metastatic ≥ 3rd line	II	**1)** Best supportive care **2)** Durvalumab + tremelimumab	180	OS
BACCI **NCT 02873195**	Advanced/ metastatic ≥ 3rd line	II	**1)** Capecitabine + bevacizumab + placebo **2)** Capecitabine + bevacizumab + atezolizumab	135	PFS
NRG Oncology **NRG-G1004** **NCT 02997228**	Advanced/ metastatic MSI-H/dMMR 1st line	III	**1)** FOLFOX + bevacizumab **2)** Atezolizumab **3)** FOLFOX + bevacizumab + atezolizumab	439	PFS
COTEZO **NCT 02788279**	Advanced/ metastatic ≥ 3rd line	III	**1)** Regorafenib **2)** Atezolizumab **3)** Atezolizumab + cobimetinib	360	OS
ALLIANCE **A021502** **NCT 02912559**	Adjuvant Stage III MSI-H/dMMR	III	**1)** FOLFOX **2)** FOLFOX + atezolizumab	720	DFS

Trial protocol / NCT ID	*Study setting*	*Phase*	*Treatment Arms*	*Planned recruitment number of patients*	*Primary outcome*
Pancreatic Cancer					
GI1616 **NCT 02866383**	Advanced/ metastatic ≥2nd line	II	**1)** Nivolumab + radiotherapy **2)** Nivolumab + ipilimumab + radiotherapy	80	Clinical benefit rate (CR + PR + SD)
NCT 02305186	Neoadjuvant	II	**1)** CRT **2)** CRT + Pembrolizumab	56	Tumour infiltrating lymphocytes in resected pancreatic tissue
NCT 02451982	Peri-operative Resectable	II	**1)** GVAX/ cyclophosphamide **2)** GVAX / cyclophosphamide + nivolumab	50	Median IL17A expression in vaccine-induced lymphoid aggregates in surgically resected pancreatic tumour
NCT 03038477	Adjuvant Neoadjuvant CRT followed by surgery	II	**1)** Observation **2)** Durvalumab	114	DFS
NCIC PA07	Advanced/ metastatic	II	**1)** Gemcitabine + nab-paclitaxel	180	OS

Trial protocol / NCT ID	*Study setting*	*Phase*	*Treatment Arms*	*Planned recruitment number of patients*	*Primary outcome*
NCT 02879318	1st line		**2)** Gemcitabine + nab-paclitaxel + durvalumab + tremelimumab		

ORR: objective response rate

DOR: duration of response

PFS: progression free survival

OS: overall survival

DFS: disease free survival

SOX: S-1 plus oxaliplatin

CAPOX: capecitabine plus oxaliplatin

FOLFOX: 5-FU, leucovorin plus oxaliplatin

FOLFIRI: 5-FU, leucovorin plus irinotecan

CRT: chemoradiation

OGJ: oesophago-gastric junction

LAG 3: lymphocyte-activation gene 3

MMP: matrix metalloproteinase

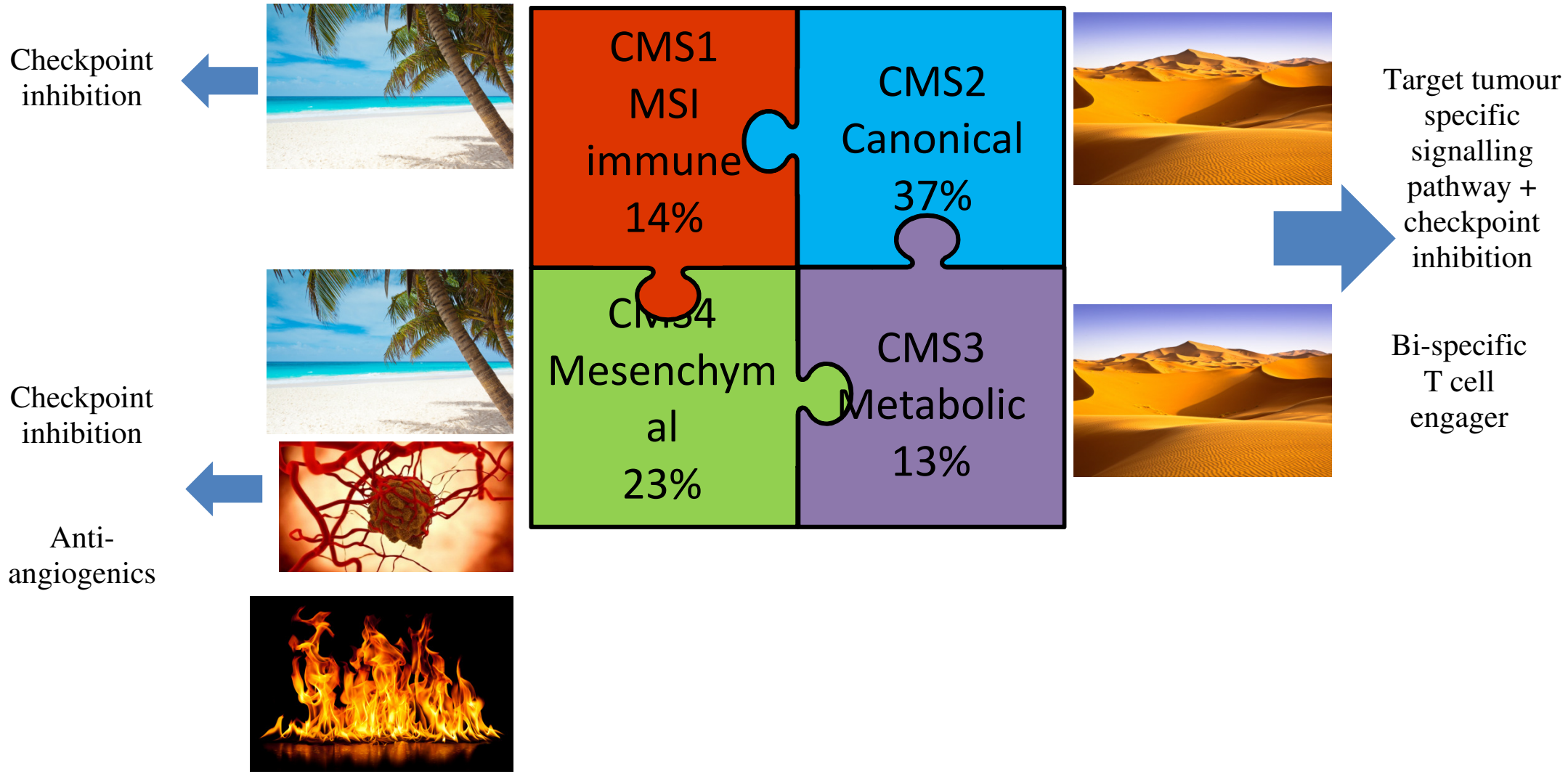
MSI-H: microsatellite instability- high

dMMR: mismatch repair deficient

FIGURE LEGEND

Figure 1 Immune landscape and consensus molecular subgroups in colorectal cancer

Figure 1


Checkpoint inhibition
Checkpoint inhibition
Anti-angiogenics
CMS1 MSI immune 14%
CMS2 Canonical 37%
CMS4 Mesenchymal 23%
CMS3 Metabolic 13%
Target tumour specific signalling pathway + checkpoint inhibition
Bi-specific T cell engager