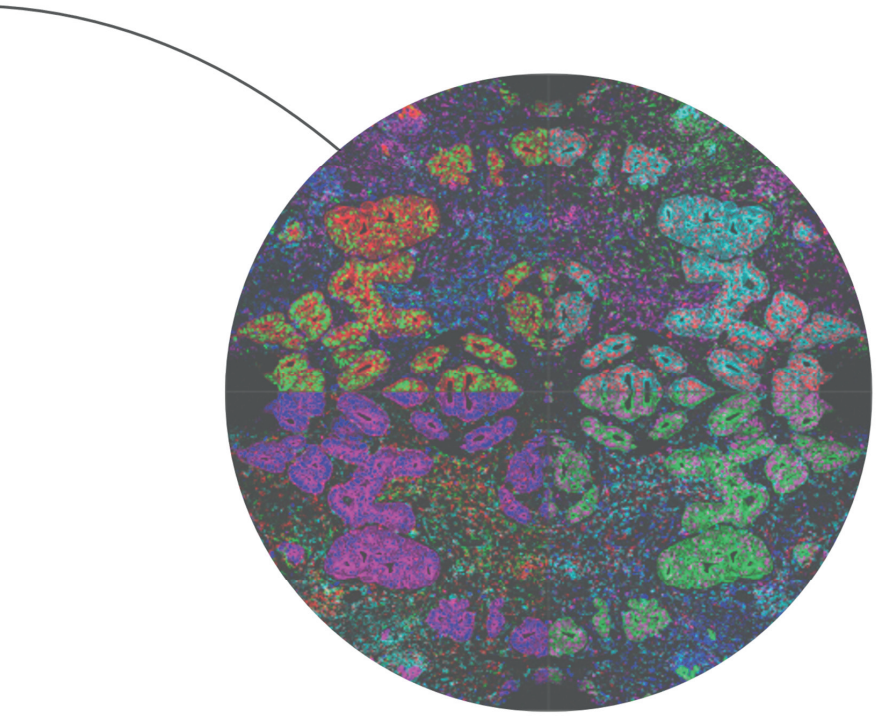
Untangling the immune landscape of colorectal cancer by in situ multiplex immunophenotyping

IJsselsteijn, M.E.



2

Colorectal cancer: a paradigmatic model for cancer immunology and immunotherapy

Published in Molecular aspects of Medicine, 2019

Marieke E. Ijsselsteijn[1], Rebeca Sanz-Pamplona[2, 3, 4], Fabienne Hermitte[5], Noel F.C.C. de Miranda[1]

[1] Department of Pathology, Leiden University Medical Center, Abinusdreef 2, 2333ZA Leiden, The Netherlands,

[2] Unit of Biomarkers and Susceptibility, Oncology Data Analytics Program (ODAP), Catalan Institute of Oncology (ICO), L'Hospitalet de Llobregat, Barcelona, Spain.

[3] Colorectal Cancer Group, ONCOBELL program, Bellvitge Biomedical Research Institute (IDIBELL), L'Hospitalet de Llobregat, Barcelona, Spain.

[4] Centro de Investigación Biomédica en Red de Epidemiologia y Salud Pública (CIBERESP), 28029 Madrid, Spain.

[5] HalioDx, 13009 Marseille, France.

Abstract

Colorectal cancer can be categorized into two major molecular subtypes according to the status of their DNA proofreading and repair machinery. The DNA repair status of tumour cells plays a major role in shaping the immune landscape of tumours and in determining the clinical response of colorectal cancer patients to immune checkpoint blockade therapies. Colorectal cancers that develop in a context of DNA mismatch repair or polymerase proofreading deficiency are generally conspicuously infiltrated by effector memory T cells and are associated with an improved clinical prognosis relative to their replication repair-proficient counterpart. While mismatch repair-deficient colorectal cancers, and most likely POLE and POLD1-mutated cancers, are amenable to immune checkpoint blockade therapies, the promise of immunotherapy still remains unfulfilled for the majority of colorectal cancer patients. This review focusses on the role of the immune system in the tumourigenesis and clinical behaviour of colorectal cancer. Furthermore, we discuss how latest advances in the fields of genomics and oncoimmunology may pave the way to broaden the scope of immunotherapy for this disease.

Keywords: Colorectal cancer; Immunotherapy; Checkpoint blockade; Mismatch repair; Neoantigens; Tumour infiltrating lymphocytes.

1. Introduction

Colorectal cancer is one of the most common and deadly cancers worldwide, estimated to have caused more than 800.000 deaths in 2018 (Bray et al., 2018). A clear distinction between colorectal cancers with high and low somatic mutation burden has provided important insights into the relationship between cancer genetics and immunity. The type and magnitude of genetic instability observed in colorectal cancer strongly influences the immune composition of the cancer microenvironment and determines the clinical responses observed to state-of-the-art cancer immunotherapies (Angelova et al., 2015; Le et al., 2017) (Figure 1). Cancers with high mutation burden (generally defined as more than 10 mutations per megabase) account for 15 to 20% of all colorectal cancers and, most often, develop in the presence of defects in the DNA mismatch repair (MMR) system, which can be inherited (Lynch syndrome) or acquired in a sporadic context. A hypermutated phenotype is also found in a minority of colorectal cancers (~1%) due to germline or somatic mutations in the genetic regions encoding the proofreading domain of DNA polymerase epsilon (POLE) or, less frequently, of DNA polymerase delta 1 (POLD1) enzymes (Cancer Genome Atlas, 2012; Rayner et al., 2016). These defects in the DNA replication and repair machinery lead to the widespread accumulation of mutations that inevitably target protein-coding, genomic regions. Moreover, nucleotide insertions and deletions are common in a background of MMR deficiency and, when targeting exonic regions, can generate frameshifted proteins with high immunogenic potential. The surplus of mutated proteins (neoantigens) in hypermutated cancers confers them an immunogenic character as demonstrated by their conspicuous infiltration with cytotoxic T cells (de Miranda et al., 2012; Dolcetti et al., 1999). Nevertheless, the large majority of colorectal cancers (up to 80% of cases) are MMR-proficient and present with low to moderate mutation burden. This review discusses how genetics and cancer immunity are closely intertwined in colorectal cancer and how this relationship impacts patient prognosis and response to state-of-the-art immunotherapies.

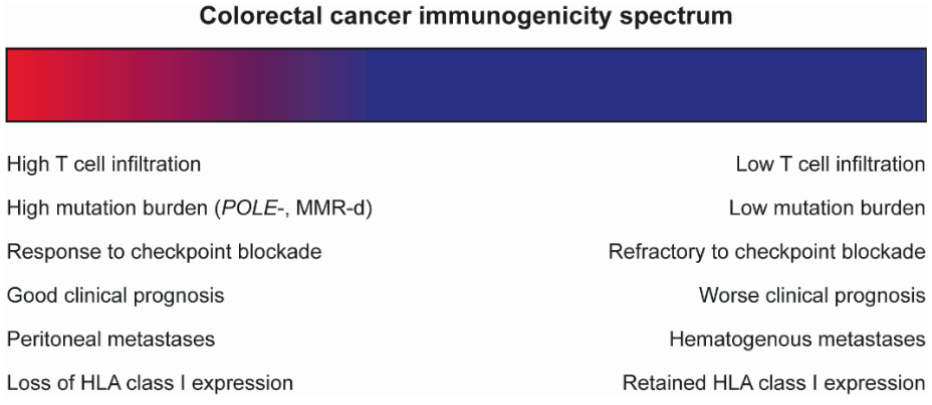


Figure 1 – In colorectal cancer, tumour mutation burden, T cell infiltration, clinical prognosis, and response to checkpoint blockade immunotherapies are closely associated. (MMR-d, Mismatch repair deficient)

2. Colorectal cancer genetics, adaptive immunity, and clinical prognosis

MMR-deficient and POLE-mutated cancers are associated with longer disease-free survival and lower risk of recurrence in stage II and III, when compared to replication repair-proficient colorectal cancers (Domingo et al., 2016), notwithstanding the decreased sensitivity of MMR-deficient tumours to fluorouracil (FU)-based adjuvant chemotherapy, a standard of care drug for the treatment of colorectal cancer (Sargent et al., 2010). On the other hand, a MMR-deficient status appears to lose its prognostic value in recurrent and metastatic disease and it has been, instead, associated with worse clinical outcomes (Alex et al., 2017; Kim et al., 2016). Interestingly, the metastatic patterns of MMR-deficient cancers are also considerably different from the remaining spectrum of colorectal cancer, with a higher frequency of peritoneal dissemination rather than hematogenous spread to liver or lungs (Fujiyoshi et al., 2017). Nevertheless, additional studies making use of large cohorts of metastatic MMR-deficient colorectal cancers are required to confirm these observations. Of note, the MMR-status also associates with other clinicopathological features that may contribute to their clinical behaviour: MMR-deficient tumours are most often diagnosed in the proximal colon while infrequent in the distal colon and rarely found in the rectum. In addition, BRAF mutations are highly prevalent in MMR-deficient colon cancers (found in approximately 50% of cases) and these tumours are further characterized by poorly differentiated and mucinous histology. (Brenner et al., 2014)

The fact that a pronounced lymphocytic infiltration is a hallmark of MMR-deficient colorectal cancers has prompted researchers to associate the immunogenic character of these tumours with their more favourable clinical behaviour. A seminal work by Galon and colleagues in 2006 confirmed the major role that the adaptive immune system plays in colorectal cancer tumourigenesis by demonstrating that the type, density, and location of immune cells in tumours had a superior prognostic value over the standard TNM staging system (Galon et al., 2006). Specifically, high T cell density was identified as an independent prognostic factor for disease-free and overall survival in patients with stage I-III colorectal cancer. Galon and colleagues went on to develop Immunoscore, a tool aimed at improving the prognostic stratification of colorectal cancer patients, particularly through the identification of high-risk stage II patients that might benefit from adjuvant therapies (Galon et al., 2012). Immunoscore categorizes tumours into low, intermediate, and high Immunoscore categories based on the assessment of CD3+ and CD8+ T cell densities at the core of tumours and their invasive margin. Recently, an international effort confirmed the value of Immunoscore as an independent prognostic tool to estimate the risk of recurrence in stage I-III colon cancer (Pages et al., 2018). Notably, in this study, tumours with a low Immunoscore and thus associated with a poor prognosis, were not restricted to MMR-proficient cancers but also observed among patients diagnosed with MMR-deficient tumours. Conversely, colon cancers with high Immunoscore were also present within the MMR-proficient subset. It would be of great interest to determine whether these nuances can be explained by fluctuations in mutational burden within MMR-deficient and -proficient subsets. It is expected that a proportion of MMR-proficient cases with a high

Immunoscore present a hypermutated phenotype resulting from mutations in the POLE and POLD1 genes. Importantly, the detailed investigation of MMR-proficient cancers which are densely infiltrated by immune cells could lead to the discovery of additional molecular mechanisms that modulate the immunogenicity of colorectal cancer.

Tumours are generally infiltrated by polyclonal populations of memory CD4+ and CD8+ T cells that display specificity to a variety of antigens; not only cancer antigens or neoantigens but also self- or viral-derived antigens (Gee et al., 2018; Scheper et al., 2019). Recent studies have proposed that cancer-specific T cells acquire discriminative phenotypes (e.g. CD103 and CD39 expression) that would support their targeted isolation for downstream therapeutic applications (Duhen et al., 2018; Simoni et al., 2018). CD103 is an integrin with affinity to E-cadherin and, thus, plays an essential role in the retention of T cells in epithelial cancers (Schon et al., 1999). Together with other markers such as CD69, CD103 identifies tissue-resident memory T cells that have been increasingly highlighted in cancer immunology studies, including colorectal cancer, as highly cytotoxic T cell populations with prognostic value (Djenidi et al., 2015; Hu et al., 2018; Webb et al., 2015). CD39 is an ectonucleotidase that, together with the ecto-5′-nucleotidase CD73, converts ADP and ATP to adenosine, a potent immunoregulatory molecule that suppresses T cell activity (Deaglio et al., 2007; Mizumoto et al., 2002). CD39+ T cells express a number of additional activation or exhaustion T cell markers (Duhen et al., 2018; Simoni et al., 2018) indicating that, in itself, CD39 might function as a checkpoint molecule that is induced after prolonged exposure of T cells to their cognate antigen. Considering the functional relevance of these phenotypes and their ability to pinpoint cancer-reactive T cells a comparison should be performed between the prognostic value of these phenotypes with the one of unselected T cell populations.

3. Other players in the colorectal cancer microenvironment

Additional immune cell subsets have been associated with the clinical behavior of colorectal cancer, including regulatory T cells (Tregs) and macrophages (Fridman et al., 2017). Tregs suppress T helper type 1 (Th1) cell-mediated responses and the activity of cytotoxic T cells but, strikingly, their presence has been associated with an improved prognosis in colorectal cancer, while predicting poor patient outcomes in other tumour types (Frey et al., 2010; Fridman et al., 2017; Salama et al., 2009). Researchers have interpreted the accumulation of Tregs in colorectal cancer as a consequence of a physiological anti-cancer immune response where the establishment of a Th1 inflammatory response is followed by the onset of suppressive mechanisms to resolve inflammation. Also, Saito and colleagues (Saito et al., 2016) proposed that the controversial role of Tregs in colorectal cancer can be explained by the existence of two distinct FOXP3+ CD4+ T cell populations with opposite functional roles: the immune suppressive activity of classical Tregs displaying high expression of the FOXP3 transcription factor was counterbalanced by the inflammatory properties of FOXP3-low, CD4+ T cells in colorectal cancer tissues. These two subpopulations

cannot be distinguished by semi-quantitative methods such as immunohistochemistry which precludes their detection in readily available retrospective colorectal cancer cohorts. Nevertheless, additional efforts will be required to address the idiosyncratic role of Tregs in colorectal cancer.

Similarly to Tregs, the role of macrophages and their impact in patient prognosis in colorectal cancer also requires clarification. Macrophages can be grossly divided into two subsets according to their phenotype and function. The so-called M1 macrophages predominantly produce proinflammatory molecules such as TNFα, nitric oxide, and reactive oxygen intermediates that can induce cancer cell-death (Mantovani et al., 2004). Conversely, M2 macrophages can arise in the context of chronic inflammation and are characterized by the production of immune suppressive, pro-angiogenic, and cell-growth factors that confer them a pro-tumourigenic role (Qian and Pollard, 2010). Tumour-associated macrophages are predominantly of the M2 subtype and their presence has been associated with a poor prognosis in a variety of cancers (Bingle et al., 2002; Qian and Pollard, 2010). However, some conflicting reports have also demonstrated that high macrophage infiltration in tumours is associated with improved patient survival, particularly in colorectal cancer (Edin et al., 2012; Forssell et al., 2007). Such discrepancies may be explained by the employment of distinct markers across studies to define macrophage populations or subsets. It is also now well-accepted that the dichotomization of macrophages into M1 and M2 subtypes is oversimplified and that these immune cells exhibit high phenotypic plasticity (Aras and Zaidi, 2017). Myeloid-derived suppressor cells (MDSC) are another population of myeloid cells with phenotypical similarities to macrophages and granulocytes but with immature features. They are proposed to result from the exposure of immature myeloid precursors to chronic inflammatory signals like the ones provided by cancer tissues (Veglia et al., 2018). They have strong immune-suppressive functions resulting from the production of factors like arginase or prostaglandin E2 and of anti-inflammatory cytokines (Huang et al., 2006; Veglia et al., 2018). Therefore, their detection in cancer patients has almost invariably been associated with poor clinical outcomes (Zhang et al., 2016). In colorectal cancer, their presence in both peripheral blood and tumour tissues has been associated with advanced tumour stages (Sun et al., 2012; Zhang et al., 2013). Furthermore, in metastatic colorectal cancer patients, high amounts of circulating MDSCs were predictive of poor progression-free survival following chemotherapy (Tada et al., 2016). Similar to macrophages, the definition of phenotypes which are discriminative of MDSC subsets has proven challenging and highly variable between studies. In the near future, it is expected that high-dimensional immunophenotyping technologies such as single-cell sequencing or multiplex imaging approaches make major contributions to advance our knowledge in this field (Finotello and Eduati, 2018; Ijsselsteijn et al., 2019a).

Although not part of the immune system, cancer-associated fibroblasts play a crucial role in the regulation of immune responses in the cancer microenvironment. The

analysis of global gene expression profiles in large cohorts of colorectal cancer has revealed four distinct consensus molecular subtypes (CMS) with biological and clinical significance (Guinney et al., 2015). The CMS1 subtype is largely composed of MMR-deficient colorectal cancers with strong immunogenic features while the CMS2 and CMS3 subtypes are associated with WNT and MYC signalling activation, and pronounced metabolic dysregulation, respectively. The CMS4 subtype, in turn, aggregates tumours with transcriptional signatures reminiscent of mesenchymal phenotypes that are driven by the TGF-β pathway. This subtype contains approximately one-fourth of all colorectal cancers and associates with worse patient survival. Importantly, the mesenchymal signatures that characterize the CMS4 subtype are not exclusively dependent on the transcriptional program of cancer cells but, instead, are strongly driven by the stromal compartment of tumours and, particularly, by cancer-associated fibroblasts (Calon et al., 2015). The reprogramming of fibroblasts through activation of the TGF-β pathway results in a positive-feedback mechanism where fibroblasts themselves become main sources of TGF-β, as well as of other immunomodulatory molecules, thereby directly suppressing the anti-tumour activity of innate and adaptive immune cell compartments (Hawinkels et al., 2014; Herrera et al., 2013; Thomas and Massague, 2005). Accordingly, the therapeutic targeting of TGF-β can have a profound impact in the augmenting of anti-tumour immune responses in colorectal cancer and other cancer models (Mariathasan et al., 2018; Tauriello et al., 2018). Furthermore, a desmoplastic stroma, composed of extracellular matrix proteins and cancer-associated fibroblasts constitutes a physical barrier that impedes the direct contact between cancer and immune cells (Elahi-Gedwillo et al., 2019). The ratio between cancer cells and stromal content has been shown to carry prognostic relevance in several solid tumours, including colorectal cancer (Huijbers et al., 2013; Vangangelt et al., 2018). Going forward, it would be of great importance that studies aimed at providing a comprehensive overview of cancer microenvironment account for the fibroblastic compartment. Further, when focusing on colorectal cancer, it is paramount to acknowledge the profound differences that distinguish MMR-deficient and MMR-proficient tumours, particularly when performing correlations with clinical parameters.

4. Immune evasion in colorectal cancer

The immune recognition of tumour cells as a result of the expression of neoantigens is highly dependent on the operationality of the antigen processing pathway in cancer cells. In this pathway, proteins are marked for degradation and are broken down into peptides by the immunoproteasome (Yewdell et al., 2003). Subsequently, these peptides are transported into the endoplasmic reticulum (ER) by transporter associated with antigen processing (TAP) proteins and loaded onto Human Leukocyte Antigen class I (HLA class I, dubbed Major Histocompatibility Complex (MHC) class I in most vertebrates) (Neefjes et al., 1993). The construction and stabilization of the HLA class I-peptide complex is facilitated by a number of chaperone proteins including calnexin, calreticulin and endoplasmic reticulum glycoprotein 57 (ERp57). These remain bound to the HLA class I complex until the loading of peptides has occurred,

as chaperoned by Tapasin (Wearsch and Cresswell, 2008). When the HLA class I-peptide complex is formed, the TAP and chaperone proteins dissociate and the HLA class I-peptide complex is transported through the Golgi apparatus to the cell surface (Neefjes et al., 2011). The exposed peptides can be directly recognized by CD8+ T cells but a competent T cell response requires the previous priming of both CD4+ and CD8+ T cells by antigen presenting cells (Kurts et al., 2010). The molecular properties of neoantigens and their affinity to the various intermediates of the antigen processing pathway, particularly to the different HLA class I alleles (Garstka et al., 2015), determines whether they will reach the cell surface in order to be presented to T cells. Furthermore, neoantigen recognition also requires that a patient's T cell repertoire includes T cells that express T cell receptors (TCRs) with affinity for specific neoantigen/HLA class I complexes (Turner et al., 2009). In line with these requirements, the accumulated evidence shows that, in cancer patients, only a minority of somatic mutations result in neoantigens that elicit a T cell response (Robbins et al., 2013; Tran et al., 2015; Verdegaal and van der Burg, 2017).

The high mutational load of proofreading- and MMR-deficient colorectal cancers, largely surpassing the threshold of 10 mutations per megabase, translates into a higher likelihood of neoantigens being recognized by patients' T cells. Moreover, these cancers are subjected to strong selective pressure to evade immune recognition. Alterations in the antigen processing pathway, leading to total loss of HLA class I expression, occur in the majority of MMR-deficient colorectal cancers (Dierssen et al., 2007; Ijsselsteijn et al., 2019b; Kloor et al., 2005). These defects are often caused by genetic aberrations in the B2M gene (Bicknell et al., 1996), coding for the HLA class I light-chain □2-microglobulin, but mutations in other components of the antigen processing machinery also explain a significant proportion of cases (Dierssen et al., 2007; Kloor et al., 2005). Furthermore, HLA class I haplotype and allelic losses can also occur and constitute more elegant mechanisms of immune escape that do not result in a "missing-self" phenotype (Dierssen et al., 2006; Karre, 1993; Maleno et al., 2004). While HLA class I phenotypical alterations have not yet been thoroughly studied in POLE- and POLD1-mutant colorectal cancers, the expectation is that they occur at similar frequencies to the ones observed in MMR-deficient cases, in line with their comparable immunogenic character. In the absence of HLA class I expression, T cell responses are most likely maintained by futile cycles of cancer cell-death, antigen take-up and presentation by antigen presenting cells, T cell priming, and failure of T cells to engage their cognate antigen at the surface of tumour cells. It is somewhat paradoxical that, despite the frequency of HLA class I alterations in MMR-deficient colorectal cancers, these cancers tend to present with a more favourable clinical outcome. Interestingly, observations from our lab and others suggest that HLA class I might be required for the establishment of colorectal cancer metastases through a hematogenous route (e.g. to the liver or lungs) (Ijsselsteijn et al., 2019b; Kloor et al., 2010). If confirmed, this would constitute a paradigmatic example on how extreme phenotypical adaptations at the primary tumour thwart the ability of cancer cells to colonize other organs, in line with the "seed and soil" model (Fidler, 2003). Conversely,

HLA class I aberrations have been described in approximately 20% of MMR-proficient colorectal cancers (Dierssen et al., 2007; Ijsselsteijn et al., 2019b; Kloor et al., 2005) but it is yet unclear whether they associate with higher mutation burden and improved prognosis in this particular subset.

The major role that the immune system plays in shaping colorectal cancer clonal evolution can also be perceived by the mutational profiles of tumours. Rooney and colleagues reported that, at the time of resection, colorectal cancers display considerably less neoantigens than the ones that would theoretically be expected in the absence of immune selection (Rooney et al., 2015). Interestingly, this effect was particularly pronounced in colorectal cancers when comparing with other tumour types.

5. Immunotherapy for colorectal cancer

Cancer immunotherapy delivered, in the last decade, a huge breakthrough in the treatment of cancer (Topalian et al., 2015). In addition to revealing the full potential of immune cells as effective therapeutic agents, it also introduced a paradigm shift from the use of targeted therapies (e.g. kinase inhibitors) that delay cancer progression to treatments with curative potential (Ribas et al., 2012). The 2018's Nobel prize laureates James Allison and Tasuku Honjo were pioneers of the cancer immunotherapy revolution (Couzin-Frankel, 2013). Their research led to the discovery of inhibitory mechanisms that regulate the activity of T cells and the clinical potential of targeting such mechanisms to treat cancer in an inspiring example of articulation between basic and translational science (Ishida et al., 1992; Leach et al., 1996). CTLA-4 and PD-1 are co-inhibitory receptors in T cells that, upon binding to their corresponding ligands (CD80/CD86 and PD-L1, respectively), inhibit T cell activity (Schwartz, 2003). This treatment strategy is particularly effective in tumours with high mutation burden as the engagement of T cells with a tumour (neo) antigen is still required for the elimination of cancer cells (Le et al., 2017; McGranahan et al., 2016; Snyder et al., 2014; Van Allen et al., 2015). In colorectal cancer, the clinical benefit of checkpoint blockade, and PD-1 blockade in particular, appears to be restricted to patients diagnosed with MMR-deficient cancers (Le et al., 2017; Le et al., 2015). Although the available clinical data is still scarce (Gong et al., 2017; Wang et al., 2018), it is expected that ongoing clinical trials (e.g. NCT03435107 and NCT03150706) also demonstrate the suitability of checkpoint blockade for the treatment of POLE- and POLD1-mutant colorectal cancers. As discussed, the occurrence of distant metastases is less common in colorectal cancer patients diagnosed with MMR-deficient tumours as compared to patients with MMR-proficient cancers. Therefore, checkpoint blockade should also be considered in case of localized disease in MMR-deficient tumours in order to maximize the number of colorectal cancer patients that can benefit from it. An exploratory trial by Chalabi et al. reported a 100% response rate to the combination of nivolumab (anti-PD-1) and ipilimumab (anti-CTLA-4) in 7 patients diagnosed with non-metastatic MMR-deficient colon cancers, in a neo-adjuvant setting (Chalabi et al., 2018).

Objective responses to PD-1 blockade can be observed in half of MMR-deficient patients with advanced disease.(Le et al., 2017; Le et al., 2015) Paradoxically, HLA class I defects have been described in up to 80% of MMR-deficient colorectal cancers which would imply that the presence of HLA class I-epitope complexes at the surface of tumour cells is not always required for therapeutic response.(Dierssen et al., 2007; Kloor et al., 2005; Middha et al., 2019) Further, and as expected, defects in antigen presentation have been identified as resistance mechanisms to checkpoint blockade therapies.(Le et al., 2017; Zaretsky et al., 2016) Nevertheless, HLA class I-independent mechanisms of response to PD-1 blockade have also been reported in other tumour types such as Hodgkin lymphoma where HLA class I loss and B2M mutations are commonly found.(Reichel et al., 2015) Greater insight into the mechanisms responsible for tumour elimination in the absence of HLA class I might reveal novel therapeutic strategies for cancers that acquired immune evasive phenotypes. Inflammatory cytokines produced during Th1 responses (e.g. IFN-γ, TNF-α) can directly contribute to HLA class I-independent cancer cell-death and growth-arrest (Balkwill, 2009; Braumuller et al., 2013). Their production can be stimulated by the (re-) activation of PD-1-expressing CD4+ and CD8+ T cells which in turn can also support the activation of innate immune cells such as NK cells, γδ T cells, or macrophages.(Marcus et al., 2014) Interestingly, recent studies have shown that these innate immune cells may constitute direct targets of PD-1 blockade.(Gordon et al., 2017; Hsu et al., 2018; Iwasaki et al., 2011) NK cells or other innate lymphoid cells expressing Killing Inhibitory Receptors (KIRs) (Wagtmann et al., 1995) are particularly attractive subsets to explore in this setting as their activity is directly inhibited by HLA class I expression at target cells.(Moretta et al., 1993) It should be noted, however, that most studies on HLA class I expression in MMR-deficient colorectal cancers were performed in primary tumours and that the frequency of HLA class I alterations might differ when investigating metastatic lesions (Ijsselsteijn et al., 2019b).

So far, immune checkpoint blockade has failed to deliver any clinical benefit to colorectal cancer patients diagnosed with MMR-proficient, low-mutation burden cancers, which constitute the majority of colorectal cancer cases (Cancer Genome Atlas, 2012; Le et al., 2015; Yarchoan et al., 2017). The near-absence of therapeutic responses in these cancers is surprising when taking into account the broad relevance of immune cell infiltration for colorectal cancer prognosis, independently of a tumour's MMR status. In the coming years, researchers should address what differentiates high T cell infiltrated MMR-deficient and -proficient colorectal cancers in their sensitivity to checkpoint blockade therapies. Interestingly, when comparing MMR-proficient colorectal cancers with other tumours carrying a similar median number of mutations per megabase, one would expect to observe clinical responses in approximately 10% of MMR-proficient colorectal cancer patients (Yarchoan et al., 2017). This discrepancy suggests that other factors, apart from mutation burden, thwart the clinical activity of checkpoint blockade antibodies in these tumours. Nevertheless, the occurrence of neoantigen-specific, tumour-infiltrating lymphocytes has been demonstrated in MMR-proficient colorectal cancers as well as other cancers with low mutation burden (Keskin

et al., 2019; Scheper et al., 2019; Tran et al., 2015; Tran et al., 2014). These observations support the development of alternative strategies that make use of these autologous, tumour-specific, immune responses (van den Bulk et al., 2018). A low number of neoantigens in MMR-deficient colorectal cancers most likely fails to provide an adequate degree of "non-selfness" that, for instance, is observed in anti-viral and -bacterial immune responses and approximated in immunogenic cancers carrying a high number of somatic mutations. This hypothesis, however, does not preclude the pursuit of strategies aimed at priming tumour-specific T cell responses or at modulating the inflammatory environment of tumours. Amongst these, the use of biomolecules corresponding to neoantigens in a therapeutic vaccination setting is a safe approach to elicit tumour-specific T cell responses (Carreno et al., 2015; Keskin et al., 2019; Ott et al., 2017). Alternatively, T cell transfer therapies can be developed based on the identification of autologous, neoantigen-specific T cells that can then be cultured in vitro and administered back to patients (Tran et al., 2016; Tran et al., 2014). While these approaches can lead to a systemic and local increase of tumour-specific T cells, they might fail to counteract the suppressive microenvironment of some tumours, dominated by Tregs, M2 macrophages, or cancer-associated fibroblasts. To counteract this, the boosting of inflammatory signals for instance by the use of oncolytic viruses (Ribas et al., 2018) or chemo-(Vincent et al., 2010) and radio-therapy (Aboudaram et al., 2017) can lead to the rewiring of the cancer microenvironment into an inflammatory profile. The latter approach might be particularly useful for rectal cancer patients that already undergo neoadjuvant treatment with chemo- and/or radio-therapy as part of the clinical management of this disease. Finally, the specific targeting of immune suppressive pathways, such as TGF-β in the CMS4 molecular subtype, is an attractive avenue to be explored in combination with checkpoint blockade therapies.

6. Concluding remarks

Colorectal cancer is a fascinating model for the study of cancer genetics and immunology. The causal association between mutational processes and anti-tumour immune responses profoundly impacts a patient's clinical prognosis and response to (immuno-) therapies (Figure 1). Fast-paced technological advances are expected to considerably deepen our knowledge of this disease in the coming years by supporting a shift of focus towards the entire genome as a source of immunogenic antigens, the discovery of novel immune cell subsets with prognostic and clinical relevance, or the development of genetic engineering approaches that allow the precise targeting of tumour cells. As we reach a plateau in the success of checkpoint blockade therapies, we can feel confident that the immune system is yet to provide novel and effective solutions for the management of colorectal cancer and other cancers.

References

Aboudaram, A., Modesto, A., Chaltiel, L., Gomez-Roca, C., Boulinguez, S., Sibaud, V., Delord, J.P., Chira, C., Delannes, M., Moyal, E., Meyer, N., 2017. Concurrent radiotherapy for patients with metastatic melanoma and receiving anti-programmed-death 1 therapy: a safe and effective combination. Melanoma Res 27 (5), 485-491.

Alex, A.K., Siqueira, S., Coudry, R., Santos, J., Alves, M., Hoff, P.M., Riechelmann, R.P., 2017. Response to Chemotherapy and Prognosis in Metastatic Colorectal Cancer With DNA Deficient Mismatch Repair. Clin Colorectal Cancer 16 (3), 228-239.

Angelova, M., Charoentong, P., Hackl, H., Fischer, M.L., Snajder, R., Krogsdam, A.M., Waldner, M.J., Bindea, G., Mlecnik, B., Galon, J., Trajanoski, Z., 2015. Characterization of the immunophenotypes and antigenomes of colorectal cancers reveals distinct tumour escape mechanisms and novel targets for immunotherapy. Genome Biol 16, 64.

Aras, S., Zaidi, M.R., 2017. TAMeless traitors: macrophages in cancer progression and metastasis. Br J Cancer 117 (11), 1583-1591.

Balkwill, F., 2009. Tumour necrosis factor and cancer. Nat Rev Cancer 9 (5), 361-371.

Bicknell, D.C., Kaklamanis, L., Hampson, R., Bodmer, W.F., Karran, P., 1996. Selection for beta 2-microglobulin mutation in mismatch repair-defective colorectal carcinomas. Curr Biol 6 (12), 1695-1697.

Bingle, L., Brown, N.J., Lewis, C.E., 2002. The role of tumour-associated macrophages in tumour progression: implications for new anticancer therapies. J Pathol 196 (3), 254-265.

Braumuller, H., Wieder, T., Brenner, E., Assmann, S., Hahn, M., Alkhaled, M., Schilbach, K., Essmann, F., Kneilling, M., Griessinger, C., Ranta, F., Ullrich, S., Mocikat, R., Braungart, K., Mehra, T., Fehrenbacher, B., Berdel, J., Niessner, H., Meier, F., van den Broek, M., Haring, H.U., Handgretinger, R., Quintanilla-Martinez, L., Fend, F., Pesic, M., Bauer, J., Zender, L., Schaller, M., Schulze-Osthoff, K., Rocken, M., 2013. T-helper-1-cell cytokines drive cancer into senescence. Nature 494 (7437), 361-365.

Bray, F., Ferlay, J., Soerjomataram, I., Siegel, R.L., Torre, L.A., Jemal, A., 2018. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 68 (6), 394-424.

Brenner, H., Kloor, M., Pox, C.P., 2014. Colorectal cancer. Lancet 383 (9927), 1490-1502.

Calon, A., Lonardo, E., Berenguer-Llergo, A., Espinet, E., Hernando-Momblona, X., Iglesias, M., Sevillano, M., Palomo-Ponce, S., Tauriello, D.V., Byrom, D., Cortina, C., Morral, C., Barcelo, C., Tosi, S., Riera, A., Attolini, C.S., Rossell, D., Sancho, E., Batlle, E., 2015. Stromal gene expression defines poor-prognosis subtypes in colorectal cancer. Nat Genet 47 (4), 320-329.

Cancer Genome Atlas, N., 2012. Comprehensive molecular characterization of human colon and rectal cancer. Nature 487 (7407), 330-337.

Carreno, B.M., Magrini, V., Becker-Hapak, M., Kaabinejadian, S., Hundal, J., Petti, A.A., Ly, A., Lie, W.R., Hildebrand, W.H., Mardis, E.R., Linette, G.P., 2015. Cancer immunotherapy. A dendritic cell vaccine increases the breadth and diversity of melanoma neoantigen-specific T cells. Science 348 (6236), 803-808.

Chalabi, M., F Fanchi, L., G Van den Berg, J., Beets, G., Lopez-Yurda, M., G Aalbers, A., Grootscholten, C., Snaebjornsson, P., Maas, M., Mertz, M., Nuijten, E., Kuiper, M., Kok, M., Leerdam, M., Schumacher, T., E Voest, E., B Haanen, J., 2018. LBA37_PRNeoadjuvant ipilimumab plus nivolumab in early stage colon cancer.

Couzin-Frankel, J., 2013. Breakthrough of the year 2013. Cancer immunotherapy. Science 342 (6165), 1432-1433.

de Miranda, N.F., Goudkade, D., Jordanova, E.S., Tops, C.M., Hes, F.J., Vasen, H.F., van Wezel, T., Morreau, H., 2012. Infiltration of Lynch colorectal cancers by activated immune cells associates with

early staging of the primary tumour and absence of lymph node metastases. Clin Cancer Res 18 (5), 1237-1245.

Deaglio, S., Dwyer, K.M., Gao, W., Friedman, D., Usheva, A., Erat, A., Chen, J.F., Enjyoji, K., Linden, J., Oukka, M., Kuchroo, V.K., Strom, T.B., Robson, S.C., 2007. Adenosine generation catalyzed by CD39 and CD73 expressed on regulatory T cells mediates immune suppression. J Exp Med 204 (6), 1257-1265.

Dierssen, J.W., de Miranda, N.F., Ferrone, S., van Puijenbroek, M., Cornelisse, C.J., Fleuren, G.J., van Wezel, T., Morreau, H., 2007. HNPCC versus sporadic microsatellite-unstable colon cancers follow different routes toward loss of HLA class I expression. BMC Cancer 7, 33.

Dierssen, J.W., de Miranda, N.F., Mulder, A., van Puijenbroek, M., Verduyn, W., Claas, F.H., van de Velde, C.J., Jan Fleuren, G., Cornelisse, C.J., Corver, W.E., Morreau, H., 2006. High-resolution analysis of HLA class I alterations in colorectal cancer. BMC Cancer 6, 233.

Djenidi, F., Adam, J., Goubar, A., Durgeau, A., Meurice, G., de Montpreville, V., Validire, P., Besse, B., Mami-Chouaib, F., 2015. CD8+CD103+ tumour-infiltrating lymphocytes are tumour-specific tissue-resident memory T cells and a prognostic factor for survival in lung cancer patients. J Immunol 194 (7), 3475-3486.

Dolcetti, R., Viel, A., Doglioni, C., Russo, A., Guidoboni, M., Capozzi, E., Vecchiato, N., Macri, E., Fornasarig, M., Boiocchi, M., 1999. High prevalence of activated intraepithelial cytotoxic T lymphocytes and increased neoplastic cell apoptosis in colorectal carcinomas with microsatellite instability. Am J Pathol 154 (6), 1805-1813.

Domingo, E., Freeman-Mills, L., Rayner, E., Glaire, M., Briggs, S., Vermeulen, L., Fessler, E., Medema, J.P., Boot, A., Morreau, H., van Wezel, T., Liefers, G.J., Lothe, R.A., Danielsen, S.A., Sveen, A., Nesbakken, A., Zlobec, I., Lugli, A., Koelzer, V.H., Berger, M.D., Castellvi-Bel, S., Munoz, J., Epicolon, c., de Bruyn, M., Nijman, H.W., Novelli, M., Lawson, K., Oukrif, D., Frangou, E., Dutton, P., Tejpar, S., Delorenzi, M., Kerr, R., Kerr, D., Tomlinson, I., Church, D.N., 2016. Somatic POLE proofreading domain mutation, immune response, and prognosis in colorectal cancer: a retrospective, pooled biomarker study. Lancet Gastroenterol Hepatol 1 (3), 207-216.

Duhen, T., Duhen, R., Montler, R., Moses, J., Moudgil, T., de Miranda, N.F., Goodall, C.P., Blair, T.C., Fox, B.A., McDermott, J.E., Chang, S.C., Grunkemeier, G., Leidner, R., Bell, R.B., Weinberg, A.D., 2018. Co-expression of CD39 and CD103 identifies tumour-reactive CD8 T cells in human solid tumours. Nat Commun 9 (1), 2724.

Edin, S., Wikberg, M.L., Dahlin, A.M., Rutegard, J., Oberg, A., Oldenborg, P.A., Palmqvist, R., 2012. The distribution of macrophages with a M1 or M2 phenotype in relation to prognosis and the molecular characteristics of colorectal cancer. PLoS One 7 (10), e47045.

Elahi-Gedwillo, K.Y., Carlson, M., Zettervall, J., Provenzano, P.P., 2019. Antifibrotic Therapy Disrupts Stromal Barriers and Modulates the Immune Landscape in Pancreatic Ductal Adenocarcinoma. Cancer Res 79 (2), 372-386.

Fidler, I.J., 2003. The pathogenesis of cancer metastasis: the 'seed and soil' hypothesis revisited. Nat Rev Cancer 3 (6), 453-458.

Finotello, F., Eduati, F., 2018. Multi-Omics Profiling of the Tumour Microenvironment: Paving the Way to Precision Immuno-Oncology. Front Oncol 8, 430.

Forssell, J., Oberg, A., Henriksson, M.L., Stenling, R., Jung, A., Palmqvist, R., 2007. High macrophage infiltration along the tumour front correlates with improved survival in colon cancer. Clin Cancer Res 13 (5), 1472-1479.

Frey, D.M., Droeser, R.A., Viehl, C.T., Zlobec, I., Lugli, A., Zingg, U., Oertli, D., Kettelhack, C., Terracciano, L., Tornillo, L., 2010. High frequency of tumour-infiltrating FOXP3(+) regulatory T cells predicts improved survival in mismatch repair-proficient colorectal cancer patients. Int J Cancer 126 (11), 2635-2643.

Fridman, W.H., Zitvogel, L., Sautes-Fridman, C., Kroemer, G., 2017. The immune contexture in cancer prognosis and treatment. Nat Rev Clin Oncol 14 (12), 717-734.

Fujiyoshi, K., Yamamoto, G., Takenoya, T., Takahashi, A., Arai, Y., Yamada, M., Kakuta, M., Yamaguchi, K., Akagi, Y., Nishimura, Y., Sakamoto, H., Akagi, K., 2017. Metastatic Pattern of Stage IV Colorectal Cancer with High-Frequency Microsatellite Instability as a Prognostic Factor. Anticancer Res 37 (1), 239-247.

Galon, J., Costes, A., Sanchez-Cabo, F., Kirilovsky, A., Mlecnik, B., Lagorce-Pages, C., Tosolini, M., Camus, M., Berger, A., Wind, P., Zinzindohoue, F., Bruneval, P., Cugnenc, P.H., Trajanoski, Z., Fridman, W.H., Pages, F., 2006. Type, density, and location of immune cells within human colorectal tumours predict clinical outcome. Science 313 (5795), 1960-1964.

Galon, J., Pages, F., Marincola, F.M., Thurin, M., Trinchieri, G., Fox, B.A., Gajewski, T.F., Ascierto, P.A., 2012. The immune score as a new possible approach for the classification of cancer. J Transl Med 10, 1.

Garstka, M.A., Fish, A., Celie, P.H., Joosten, R.P., Janssen, G.M., Berlin, I., Hoppes, R., Stadnik, M., Janssen, L., Ovaa, H., van Veelen, P.A., Perrakis, A., Neefjes, J., 2015. The first step of peptide selection in antigen presentation by MHC class I molecules. Proc Natl Acad Sci U S A 112 (5), 1505-1510.

Gee, M.H., Han, A., Lofgren, S.M., Beausang, J.F., Mendoza, J.L., Birnbaum, M.E., Bethune, M.T., Fischer, S., Yang, X., Gomez-Eerland, R., Bingham, D.B., Sibener, L.V., Fernandes, R.A., Velasco, A., Baltimore, D., Schumacher, T.N., Khatri, P., Quake, S.R., Davis, M.M., Garcia, K.C., 2018. Antigen Identification for Orphan T Cell Receptors Expressed on Tumour-Infiltrating Lymphocytes. Cell 172 (3), 549-563 e516.

Gong, J., Wang, C., Lee, P.P., Chu, P., Fakih, M., 2017. Response to PD-1 Blockade in Microsatellite Stable Metastatic Colorectal Cancer Harboring a POLE Mutation. J Natl Compr Canc Netw 15 (2), 142-147.

Gordon, S.R., Maute, R.L., Dulken, B.W., Hutter, G., George, B.M., McCracken, M.N., Gupta, R., Tsai, J.M., Sinha, R., Corey, D., Ring, A.M., Connolly, A.J., Weissman, I.L., 2017. PD-1 expression by tumour-associated macrophages inhibits phagocytosis and tumour immunity. Nature 545 (7655), 495-499.

Guinney, J., Dienstmann, R., Wang, X., de Reynies, A., Schlicker, A., Soneson, C., Marisa, L., Roepman, P., Nyamundanda, G., Angelino, P., Bot, B.M., Morris, J.S., Simon, I.M., Gerster, S., Fessler, E., De Sousa, E.M.F., Missiaglia, E., Ramay, H., Barras, D., Homicsko, K., Maru, D., Manyam, G.C., Broom, B., Boige, V., Perez-Villamil, B., Laderas, T., Salazar, R., Gray, J.W., Hanahan, D., Tabernero, J., Bernards, R., Friend, S.H., Laurent-Puig, P., Medema, J.P., Sadanandam, A., Wessels, L., Delorenzi, M., Kopetz, S., Vermeulen, L., Tejpar, S., 2015. The consensus molecular subtypes of colorectal cancer. Nat Med 21 (11), 1350-1356.

Hawinkels, L.J., Paauwe, M., Verspaget, H.W., Wiercinska, E., van der Zon, J.M., van der Ploeg, K., Koelink, P.J., Lindeman, J.H., Mesker, W., ten Dijke, P., Sier, C.F., 2014. Interaction with colon cancer cells hyperactivates TGF-beta signaling in cancer-associated fibroblasts. Oncogene 33 (1), 97-107.

Herrera, M., Herrera, A., Dominguez, G., Silva, J., Garcia, V., Garcia, J.M., Gomez, I., Soldevilla, B., Munoz, C., Provencio, M., Campos-Martin, Y., Garcia de Herreros, A., Casal, I., Bonilla, F., Pena, C., 2013. Cancer-associated fibroblast and M2 macrophage markers together predict outcome in colorectal cancer patients. Cancer Sci 104 (4), 437-444.

Hsu, J., Hodgins, J.J., Marathe, M., Nicolai, C.J., Bourgeois-Daigneault, M.C., Trevino, T.N., Azimi, C.S., Scheer, A.K., Randolph, H.E., Thompson, T.W., Zhang, L., Iannello, A., Mathur, N., Jardine, K.E., Kirn, G.A., Bell, J.C., McBurney, M.W., Raulet, D.H., Ardolino, M., 2018. Contribution of NK cells to immunotherapy mediated by PD-1/PD-L1 blockade. J Clin Invest 128 (10), 4654-4668.

Hu, X., Li, Y.Q., Li, Q.G., Ma, Y.L., Peng, J.J., Cai, S.J., 2018. ITGAE Defines CD8+ Tumour-Infiltrating Lymphocytes Predicting a better Prognostic Survival in Colorectal Cancer. EBioMedicine 35, 178-188.

Huang, B., Pan, P.Y., Li, Q., Sato, A.I., Levy, D.E., Bromberg, J., Divino, C.M., Chen, S.H., 2006. Gr-1+CD115+ immature myeloid suppressor cells mediate the development of tumour-induced T regulatory cells and T-cell anergy in tumour-bearing host. Cancer Res 66 (2), 1123-1131.

Huijbers, A., Tollenaar, R.A., v Pelt, G.W., Zeestraten, E.C., Dutton, S., McConkey, C.C., Domingo, E., Smit, V.T., Midgley, R., Warren, B.F., Johnstone, E.C., Kerr, D.J., Mesker, W.E., 2013. The proportion of tumour-stroma as a strong prognosticator for stage II and III colon cancer patients: validation in the VICTOR trial. Ann Oncol 24 (1), 179-185.

Ijsselsteijn, M.E., Brouwer, T.P., Abdulrahman, Z., Reidy, E., Ramalheiro, A., Heeren, A.M., Vahrmeijer, A., Jordanova, E.S., de Miranda, N.F., 2019a. Cancer immunophenotyping by seven-colour multispectral imaging without tyramide signal amplification. J Pathol Clin Res 5 (1), 3-11.

Ijsselsteijn, M.E., Petitprez, F., Lacroix, L., Ruano, D., van der Breggen, R., Julie, C., Morreau, H., Sautes-Fridman, C., Fridman, W.H., de Miranda, N., 2019b. Revisiting immune escape in colorectal cancer in the era of immunotherapy. Br J Cancer.

Ishida, Y., Agata, Y., Shibahara, K., Honjo, T., 1992. Induced expression of PD-1, a novel member of the immunoglobulin gene superfamily, upon programmed cell death. EMBO J 11 (11), 3887-3895.

Iwasaki, M., Tanaka, Y., Kobayashi, H., Murata-Hirai, K., Miyabe, H., Sugie, T., Toi, M., Minato, N., 2011. Expression and function of PD-1 in human gammadelta T cells that recognize phosphoantigens. Eur J Immunol 41 (2), 345-355.

Karre, K., 1993. Natural killer cells and the MHC class I pathway of peptide presentation. Semin Immunol 5 (2), 127-145.

Keskin, D.B., Anandappa, A.J., Sun, J., Tirosh, I., Mathewson, N.D., Li, S., Oliveira, G., Giobbie-Hurder, A., Felt, K., Gjini, E., Shukla, S.A., Hu, Z., Li, L., Le, P.M., Allesoe, R.L., Richman, A.R., Kowalczyk, M.S., Abdelrahman, S., Geduldig, J.E., Charbonneau, S., Pelton, K., Iorgulescu, J.B., Elagina, L., Zhang, W., Olive, O., McCluskey, C., Olsen, L.R., Stevens, J., Lane, W.J., Salazar, A.M., Daley, H., Wen, P.Y., Chiocca, E.A., Harden, M., Lennon, N.J., Gabriel, S., Getz, G., Lander, E.S., Regev, A., Ritz, J., Neuberg, D., Rodig, S.J., Ligon, K.L., Suva, M.L., Wucherpfennig, K.W., Hacohen, N., Fritsch, E.F., Livak, K.J., Ott, P.A., Wu, C.J., Reardon, D.A., 2019. Neoantigen vaccine generates intratumoural T cell responses in phase Ib glioblastoma trial. Nature 565 (7738), 234-239.

Kim, C.G., Ahn, J.B., Jung, M., Beom, S.H., Kim, C., Kim, J.H., Heo, S.J., Park, H.S., Kim, J.H., Kim, N.K., Min, B.S., Kim, H., Koom, W.S., Shin, S.J., 2016. Effects of microsatellite instability on recurrence patterns and outcomes in colorectal cancers. Br J Cancer 115 (1), 25-33.

Kloor, M., Becker, C., Benner, A., Woerner, S.M., Gebert, J., Ferrone, S., von Knebel Doeberitz, M., 2005. Immunoselective pressure and human leukocyte antigen class I antigen machinery defects in microsatellite unstable colorectal cancers. Cancer Res 65 (14), 6418-6424.

Kloor, M., Michel, S., von Knebel Doeberitz, M., 2010. Immune evasion of microsatellite unstable colorectal cancers. Int J Cancer 127 (5), 1001-1010.

Kurts, C., Robinson, B.W., Knolle, P.A., 2010. Cross-priming in health and disease. Nat Rev Immunol 10 (6), 403-414.

Le, D.T., Durham, J.N., Smith, K.N., Wang, H., Bartlett, B.R., Aulakh, L.K., Lu, S., Kemberling, H., Wilt, C., Luber, B.S., Wong, F., Azad, N.S., Rucki, A.A., Laheru, D., Donehower, R., Zaheer, A., Fisher, G.A., Crocenzi, T.S., Lee, J.J., Greten, T.F., Duffy, A.G., Ciombor, K.K., Eyring, A.D., Lam, B.H., Joe, A., Kang, S.P., Holdhoff, M., Danilova, L., Cope, L., Meyer, C., Zhou, S., Goldberg, R.M., Armstrong, D.K., Bever, K.M., Fader, A.N., Taube, J., Housseau, F., Spetzler, D., Xiao, N., Pardoll, D.M., Papadopoulos, N., Kinzler, K.W., Eshleman, J.R., Vogelstein, B., Anders, R.A., Diaz, L.A., Jr., 2017. Mismatch repair deficiency predicts response of solid tumours to PD-1 blockade. Science 357 (6349), 409-413.

Le, D.T., Uram, J.N., Wang, H., Bartlett, B.R., Kemberling, H., Eyring, A.D., Skora, A.D., Luber, B.S., Azad, N.S., Laheru, D., Biedrzycki, B., Donehower, R.C., Zaheer, A., Fisher, G.A., Crocenzi, T.S., Lee, J.J., Duffy, S.M., Goldberg, R.M., de la Chapelle, A., Koshiji, M., Bhaijee, F., Huebner, T., Hruban, R.H., Wood, L.D., Cuka, N., Pardoll, D.M., Papadopoulos, N., Kinzler, K.W., Zhou, S., Cornish, T.C., Taube,

J.M., Anders, R.A., Eshleman, J.R., Vogelstein, B., Diaz, L.A., Jr., 2015. PD-1 Blockade in Tumours with Mismatch-Repair Deficiency. N Engl J Med 372 (26), 2509-2520.

Leach, D.R., Krummel, M.F., Allison, J.P., 1996. Enhancement of antitumour immunity by CTLA-4 blockade. Science 271 (5256), 1734-1736.

Maleno, I., Cabrera, C.M., Cabrera, T., Paco, L., Lopez-Nevot, M.A., Collado, A., Ferron, A., Garrido, F., 2004. Distribution of HLA class I altered phenotypes in colorectal carcinomas: high frequency of HLA haplotype loss associated with loss of heterozygosity in chromosome region 6p21. Immunogenetics 56 (4), 244-253.

Mantovani, A., Sica, A., Sozzani, S., Allavena, P., Vecchi, A., Locati, M., 2004. The chemokine system in diverse forms of macrophage activation and polarization. Trends Immunol 25 (12), 677-686.

Marcus, A., Gowen, B.G., Thompson, T.W., Iannello, A., Ardolino, M., Deng, W., Wang, L., Shifrin, N., Raulet, D.H., 2014. Recognition of tumours by the innate immune system and natural killer cells. Adv Immunol 122, 91-128.

Mariathasan, S., Turley, S.J., Nickles, D., Castiglioni, A., Yuen, K., Wang, Y., Kadel, E.E., III, Koeppen, H., Astarita, J.L., Cubas, R., Jhunjhunwala, S., Banchereau, R., Yang, Y., Guan, Y., Chalouni, C., Ziai, J., Senbabaoglu, Y., Santoro, S., Sheinson, D., Hung, J., Giltnane, J.M., Pierce, A.A., Mesh, K., Lianoglou, S., Riegler, J., Carano, R.A.D., Eriksson, P., Hoglund, M., Somarriba, L., Halligan, D.L., van der Heijden, M.S., Loriot, Y., Rosenberg, J.E., Fong, L., Mellman, I., Chen, D.S., Green, M., Derleth, C., Fine, G.D., Hegde, P.S., Bourgon, R., Powles, T., 2018. TGFbeta attenuates tumour response to PD-L1 blockade by contributing to exclusion of T cells. Nature 554 (7693), 544-548.

McGranahan, N., Furness, A.J., Rosenthal, R., Ramskov, S., Lyngaa, R., Saini, S.K., Jamal-Hanjani, M., Wilson, G.A., Birkbak, N.J., Hiley, C.T., Watkins, T.B., Shafi, S., Murugaesu, N., Mitter, R., Akarca, A.U., Linares, J., Marafioti, T., Henry, J.Y., Van Allen, E.M., Miao, D., Schilling, B., Schadendorf, D., Garraway, L.A., Makarov, V., Rizvi, N.A., Snyder, A., Hellmann, M.D., Merghoub, T., Wolchok, J.D., Shukla, S.A., Wu, C.J., Peggs, K.S., Chan, T.A., Hadrup, S.R., Quezada, S.A., Swanton, C., 2016. Clonal neoantigens elicit T cell immunoreactivity and sensitivity to immune checkpoint blockade. Science 351 (6280), 1463-1469.

Middha, S., Yaeger, R., Shia, J., Stadler, Z.K., King, S., Guercio, S., Paroder, V., Bates, D.D.B., Rana, S., Diaz, L.A., Jr., Saltz, L., Segal, N., Ladanyi, M., Zehir, A., Hechtman, J.F., 2019. Majority of B2M-Mutant and -Deficient Colorectal Carcinomas Achieve Clinical Benefit From Immune Checkpoint Inhibitor Therapy and Are Microsatellite Instability-High. JCO Precis Oncol 3.

Mizumoto, N., Kumamoto, T., Robson, S.C., Sevigny, J., Matsue, H., Enjyoji, K., Takashima, A., 2002. CD39 is the dominant Langerhans cell-associated ecto-NTPDase: modulatory roles in inflammation and immune responsiveness. Nat Med 8 (4), 358-365.

Moretta, A., Vitale, M., Bottino, C., Orengo, A.M., Morelli, L., Augugliaro, R., Barbaresi, M., Ciccone, E., Moretta, L., 1993. P58 molecules as putative receptors for major histocompatibility complex (MHC) class I molecules in human natural killer (NK) cells. Anti-p58 antibodies reconstitute lysis of MHC class I-protected cells in NK clones displaying different specificities. J Exp Med 178 (2), 597-604.

Neefjes, J., Jongsma, M.L., Paul, P., Bakke, O., 2011. Towards a systems understanding of MHC class I and MHC class II antigen presentation. Nat Rev Immunol 11 (12), 823-836.

Neefjes, J.J., Momburg, F., Hammerling, G.J., 1993. Selective and ATP-dependent translocation of peptides by the MHC-encoded transporter. Science 261 (5122), 769-771.

Ott, P.A., Hu, Z., Keskin, D.B., Shukla, S.A., Sun, J., Bozym, D.J., Zhang, W., Luoma, A., Giobbie-Hurder, A., Peter, L., Chen, C., Olive, O., Carter, T.A., Li, S., Lieb, D.J., Eisenhaure, T., Gjini, E., Stevens, J., Lane, W.J., Javeri, I., Nellaiappan, K., Salazar, A.M., Daley, H., Seaman, M., Buchbinder, E.I., Yoon, C.H., Harden, M., Lennon, N., Gabriel, S., Rodig, S.J., Barouch, D.H., Aster, J.C., Getz, G., Wucherpfennig, K., Neuberg, D., Ritz, J., Lander, E.S., Fritsch, E.F., Hacohen, N., Wu, C.J., 2017. An immunogenic personal neoantigen vaccine for patients with melanoma. Nature 547 (7662), 217-221.

Pages, F., Mlecnik, B., Marliot, F., Bindea, G., Ou, F.S., Bifulco, C., Lugli, A., Zlobec, I., Rau, T.T., Berger, M.D., Nagtegaal, I.D., Vink-Borger, E., Hartmann, A., Geppert, C., Kolwelter, J., Merkel, S., Grutzmann, R., Van den Eynde, M., Jouret-Mourin, A., Kartheuser, A., Leonard, D., Remue, C., Wang, J.Y., Bavi, P., Roehrl, M.H.A., Ohashi, P.S., Nguyen, L.T., Han, S., MacGregor, H.L., Hafezi-Bakhtiari, S., Wouters, B.G., Masucci, G.V., Andersson, E.K., Zavadova, E., Vocka, M., Spacek, J., Petruzelka, L., Konopasek, B., Dundr, P., Skalova, H., Nemejcova, K., Botti, G., Tatangelo, F., Delrio, P., Ciliberto, G., Maio, M., Laghi, L., Grizzi, F., Fredriksen, T., Buttard, B., Angelova, M., Vasaturo, A., Maby, P., Church, S.E., Angell, H.K., Lafontaine, L., Bruni, D., El Sissy, C., Haicheur, N., Kirilovsky, A., Berger, A., Lagorce, C., Meyers, J.P., Paustian, C., Feng, Z., Ballesteros-Merino, C., Dijkstra, J., van de Water, C., van Lent-van Vliet, S., Knijn, N., Musina, A.M., Scripcariu, D.V., Popivanova, B., Xu, M., Fujita, T., Hazama, S., Suzuki, N., Nagano, H., Okuno, K., Torigoe, T., Sato, N., Furuhata, T., Takemasa, I., Itoh, K., Patel, P.S., Vora, H.H., Shah, B., Patel, J.B., Rajvik, K.N., Pandya, S.J., Shukla, S.N., Wang, Y., Zhang, G., Kawakami, Y., Marincola, F.M., Ascierto, P.A., Sargent, D.J., Fox, B.A., Galon, J., 2018. International validation of the consensus Immunoscore for the classification of colon cancer: a prognostic and accuracy study. Lancet 391 (10135), 2128-2139.

Qian, B.Z., Pollard, J.W., 2010. Macrophage diversity enhances tumour progression and metastasis. Cell 141 (1), 39-51.

Rayner, E., van Gool, I.C., Palles, C., Kearsey, S.E., Bosse, T., Tomlinson, I., Church, D.N., 2016. A panoply of errors: polymerase proofreading domain mutations in cancer. Nat Rev Cancer 16 (2), 71-81.

Reichel, J., Chadburn, A., Rubinstein, P.G., Giulino-Roth, L., Tam, W., Liu, Y., Gaiolla, R., Eng, K., Brody, J., Inghirami, G., Carlo-Stella, C., Santoro, A., Rahal, D., Totonchy, J., Elemento, O., Cesarman, E., Roshal, M., 2015. Flow sorting and exome sequencing reveal the oncogenome of primary Hodgkin and Reed-Sternberg cells. Blood 125 (7), 1061-1072.

Ribas, A., Dummer, R., Puzanov, I., VanderWalde, A., Andtbacka, R.H.I., Michielin, O., Olszanski, A.J., Malvehy, J., Cebon, J., Fernandez, E., Kirkwood, J.M., Gajewski, T.F., Chen, L., Gorski, K.S., Anderson, A.A., Diede, S.J., Lassman, M.E., Gansert, J., Hodi, F.S., Long, G.V., 2018. Oncolytic Virotherapy Promotes Intratumoural T Cell Infiltration and Improves Anti-PD-1 Immunotherapy. Cell 174 (4), 1031-1032.

Ribas, A., Hersey, P., Middleton, M.R., Gogas, H., Flaherty, K.T., Sondak, V.K., Kirkwood, J.M., 2012. New challenges in endpoints for drug development in advanced melanoma. Clin Cancer Res 18 (2), 336-341.

Robbins, P.F., Lu, Y.C., El-Gamil, M., Li, Y.F., Gross, C., Gartner, J., Lin, J.C., Teer, J.K., Cliften, P., Tycksen, E., Samuels, Y., Rosenberg, S.A., 2013. Mining exomic sequencing data to identify mutated antigens recognized by adoptively transferred tumour-reactive T cells. Nat Med 19 (6), 747-752.

Rooney, M.S., Shukla, S.A., Wu, C.J., Getz, G., Hacohen, N., 2015. Molecular and genetic properties of tumours associated with local immune cytolytic activity. Cell 160 (1-2), 48-61.

Saito, T., Nishikawa, H., Wada, H., Nagano, Y., Sugiyama, D., Atarashi, K., Maeda, Y., Hamaguchi, M., Ohkura, N., Sato, E., Nagase, H., Nishimura, J., Yamamoto, H., Takiguchi, S., Tanoue, T., Suda, W., Morita, H., Hattori, M., Honda, K., Mori, M., Doki, Y., Sakaguchi, S., 2016. Two FOXP3(+)CD4(+) T cell subpopulations distinctly control the prognosis of colorectal cancers. Nat Med 22 (6), 679-684.

Salama, P., Phillips, M., Grieu, F., Morris, M., Zeps, N., Joseph, D., Platell, C., Iacopetta, B., 2009. Tumour-infiltrating FOXP3+ T regulatory cells show strong prognostic significance in colorectal cancer. J Clin Oncol 27 (2), 186-192.

Sargent, D.J., Marsoni, S., Monges, G., Thibodeau, S.N., Labianca, R., Hamilton, S.R., French, A.J., Kabat, B., Foster, N.R., Torri, V., Ribic, C., Grothey, A., Moore, M., Zaniboni, A., Seitz, J.F., Sinicrope, F., Gallinger, S., 2010. Defective mismatch repair as a predictive marker for lack of efficacy of fluorouracil-based adjuvant therapy in colon cancer. J Clin Oncol 28 (20), 3219-3226.

Scheper, W., Kelderman, S., Fanchi, L.F., Linnemann, C., Bendle, G., de Rooij, M.A.J., Hirt, C., Mezzadra, R., Slagter, M., Dijkstra, K., Kluin, R.J.C., Snaebjornsson, P., Milne, K., Nelson, B.H., Zijlmans, H., Kenter, G., Voest, E.E., Haanen, J., Schumacher, T.N., 2019. Low and variable tumour reactivity of the intratumoural TCR repertoire in human cancers. Nat Med 25 (1), 89-94.

Schon, M.P., Arya, A., Murphy, E.A., Adams, C.M., Strauch, U.G., Agace, W.W., Marsal, J., Donohue, J.P., Her, H., Beier, D.R., Olson, S., Lefrancois, L., Brenner, M.B., Grusby, M.J., Parker, C.M., 1999. Mucosal T lymphocyte numbers are selectively reduced in integrin alpha E (CD103)-deficient mice. J Immunol 162 (11), 6641-6649.

Schwartz, R.H., 2003. T cell anergy. Annu Rev Immunol 21, 305-334.

Simoni, Y., Becht, E., Fehlings, M., Loh, C.Y., Koo, S.L., Teng, K.W.W., Yeong, J.P.S., Nahar, R., Zhang, T., Kared, H., Duan, K., Ang, N., Poidinger, M., Lee, Y.Y., Larbi, A., Khng, A.J., Tan, E., Fu, C., Mathew, R., Teo, M., Lim, W.T., Toh, C.K., Ong, B.H., Koh, T., Hillmer, A.M., Takano, A., Lim, T.K.H., Tan, E.H., Zhai, W., Tan, D.S.W., Tan, I.B., Newell, E.W., 2018. Bystander CD8(+) T cells are abundant and phenotypically distinct in human tumour infiltrates. Nature 557 (7706), 575-579.

Snyder, A., Makarov, V., Merghoub, T., Yuan, J., Zaretsky, J.M., Desrichard, A., Walsh, L.A., Postow, M.A., Wong, P., Ho, T.S., Hollmann, T.J., Bruggeman, C., Kannan, K., Li, Y., Elipenahli, C., Liu, C., Harbison, C.T., Wang, L., Ribas, A., Wolchok, J.D., Chan, T.A., 2014. Genetic basis for clinical response to CTLA-4 blockade in melanoma. N Engl J Med 371 (23), 2189-2199.

Sun, H.L., Zhou, X., Xue, Y.F., Wang, K., Shen, Y.F., Mao, J.J., Guo, H.F., Miao, Z.N., 2012. Increased frequency and clinical significance of myeloid-derived suppressor cells in human colorectal carcinoma. World J Gastroenterol 18 (25), 3303-3309.

Tada, K., Kitano, S., Shoji, H., Nishimura, T., Shimada, Y., Nagashima, K., Aoki, K., Hiraoka, N., Honma, Y., Iwasa, S., Okita, N., Takashima, A., Kato, K., Yamada, Y., Katayama, N., Boku, N., Heike, Y., Hamaguchi, T., 2016. Pretreatment Immune Status Correlates with Progression-Free Survival in Chemotherapy-Treated Metastatic Colorectal Cancer Patients. Cancer Immunol Res 4 (7), 592-599.

Tauriello, D.V.F., Palomo-Ponce, S., Stork, D., Berenguer-Llergo, A., Badia-Ramentol, J., Iglesias, M., Sevillano, M., Ibiza, S., Canellas, A., Hernando-Momblona, X., Byrom, D., Matarin, J.A., Calon, A., Rivas, E.I., Nebreda, A.R., Riera, A., Attolini, C.S., Batlle, E., 2018. TGFbeta drives immune evasion in genetically reconstituted colon cancer metastasis. Nature 554 (7693), 538-543.

Thomas, D.A., Massague, J., 2005. TGF-beta directly targets cytotoxic T cell functions during tumour evasion of immune surveillance. Cancer Cell 8 (5), 369-380.

Topalian, S.L., Drake, C.G., Pardoll, D.M., 2015. Immune checkpoint blockade: a common denominator approach to cancer therapy. Cancer Cell 27 (4), 450-461.

Tran, E., Ahmadzadeh, M., Lu, Y.C., Gros, A., Turcotte, S., Robbins, P.F., Gartner, J.J., Zheng, Z., Li, Y.F., Ray, S., Wunderlich, J.R., Somerville, R.P., Rosenberg, S.A., 2015. Immunogenicity of somatic mutations in human gastrointestinal cancers. Science 350 (6266), 1387-1390.

Tran, E., Robbins, P.F., Lu, Y.C., Prickett, T.D., Gartner, J.J., Jia, L., Pasetto, A., Zheng, Z., Ray, S., Groh, E.M., Kriley, I.R., Rosenberg, S.A., 2016. T-Cell Transfer Therapy Targeting Mutant KRAS in Cancer. N Engl J Med 375 (23), 2255-2262.

Tran, E., Turcotte, S., Gros, A., Robbins, P.F., Lu, Y.C., Dudley, M.E., Wunderlich, J.R., Somerville, R.P., Hogan, K., Hinrichs, C.S., Parkhurst, M.R., Yang, J.C., Rosenberg, S.A., 2014. Cancer immunotherapy based on mutation-specific CD4+ T cells in a patient with epithelial cancer. Science 344 (6184), 641-645.

Turner, S.J., La Gruta, N.L., Kedzierska, K., Thomas, P.G., Doherty, P.C., 2009. Functional implications of T cell receptor diversity. Curr Opin Immunol 21 (3), 286-290.

Van Allen, E.M., Miao, D., Schilling, B., Shukla, S.A., Blank, C., Zimmer, L., Sucker, A., Hillen, U., Foppen, M.H.G., Goldinger, S.M., Utikal, J., Hassel, J.C., Weide, B., Kaehler, K.C., Loquai, C., Mohr, P., Gutzmer, R., Dummer, R., Gabriel, S., Wu, C.J., Schadendorf, D., Garraway, L.A., 2015. Genomic correlates of response to CTLA-4 blockade in metastatic melanoma. Science 350 (6257), 207-211.

van den Bulk, J., Verdegaal, E.M., de Miranda, N.F., 2018. Cancer immunotherapy: broadening the scope of targetable tumours. Open Biol 8 (6).

Vangangelt, K.M.H., van Pelt, G.W., Engels, C.C., Putter, H., Liefers, G.J., Smit, V., Tollenaar, R., Kuppen, P.J.K., Mesker, W.E., 2018. Prognostic value of tumour-stroma ratio combined with the immune status of tumours in invasive breast carcinoma. Breast Cancer Res Treat 168 (3), 601-612.

Veglia, F., Perego, M., Gabrilovich, D., 2018. Myeloid-derived suppressor cells coming of age. Nat Immunol 19 (2), 108-119.

Verdegaal, E.M.E., van der Burg, S.H., 2017. The Potential and Challenges of Exploiting the Vast But Dynamic Neoepitope Landscape for Immunotherapy. Front Immunol 8, 1113.

Vincent, J., Mignot, G., Chalmin, F., Ladoire, S., Bruchard, M., Chevriaux, A., Martin, F., Apetoh, L., Rebe, C., Ghiringhelli, F., 2010. 5-Fluorouracil selectively kills tumour-associated myeloid-derived suppressor cells resulting in enhanced T cell-dependent antitumour immunity. Cancer Res 70 (8), 3052-3061.

Wagtmann, N., Biassoni, R., Cantoni, C., Verdiani, S., Malnati, M.S., Vitale, M., Bottino, C., Moretta, L., Moretta, A., Long, E.O., 1995. Molecular clones of the p58 NK cell receptor reveal immunoglobulin-related molecules with diversity in both the extra- and intracellular domains. Immunity 2 (5), 439-449.

Wang, C., Gong, J., Tu, T.Y., Lee, P.P., Fakih, M., 2018. Immune profiling of microsatellite instability-high and polymerase epsilon (POLE)-mutated metastatic colorectal tumours identifies predictors of response to anti-PD-1 therapy. J Gastrointest Oncol 9 (3), 404-415.

Wearsch, P.A., Cresswell, P., 2008. The quality control of MHC class I peptide loading. Curr Opin Cell Biol 20 (6), 624-631.

Webb, J.R., Milne, K., Nelson, B.H., 2015. PD-1 and CD103 Are Widely Coexpressed on Prognostically Favorable Intraepithelial CD8 T Cells in Human Ovarian Cancer. Cancer Immunol Res 3 (8), 926-935.

Yarchoan, M., Hopkins, A., Jaffee, E.M., 2017. Tumour Mutational Burden and Response Rate to PD-1 Inhibition. N Engl J Med 377 (25), 2500-2501.

Yewdell, J.W., Reits, E., Neefjes, J., 2003. Making sense of mass destruction: quantitating MHC class I antigen presentation. Nat Rev Immunol 3 (12), 952-961.

Zaretsky, J.M., Garcia-Diaz, A., Shin, D.S., Escuin-Ordinas, H., Hugo, W., Hu-Lieskovan, S., Torrejon, D.Y., Abril-Rodriguez, G., Sandoval, S., Barthly, L., Saco, J., Homet Moreno, B., Mezzadra, R., Chmielowski, B., Ruchalski, K., Shintaku, I.P., Sanchez, P.J., Puig-Saus, C., Cherry, G., Seja, E., Kong, X., Pang, J., Berent-Maoz, B., Comin-Anduix, B., Graeber, T.G., Tumeh, P.C., Schumacher, T.N., Lo, R.S., Ribas, A., 2016. Mutations Associated with Acquired Resistance to PD-1 Blockade in Melanoma. N Engl J Med 375 (9), 819-829.

Zhang, B., Wang, Z., Wu, L., Zhang, M., Li, W., Ding, J., Zhu, J., Wei, H., Zhao, K., 2013. Circulating and tumour-infiltrating myeloid-derived suppressor cells in patients with colorectal carcinoma. PLoS One 8 (2), e57114.

Zhang, S., Ma, X., Zhu, C., Liu, L., Wang, G., Yuan, X., 2016. The Role of Myeloid-Derived Suppressor Cells in Patients with Solid Tumours: A Meta-Analysis. PLoS One 11 (10), e0164514.