



## Cancer immune contexture and immunotherapy

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1 **Cancer immune contexture and immunotherapy**

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9

## 10 **Abstract**

11 The immune contexture characterizes the clinical impact of the density, the location,  
12 the organization and the functional orientation of tumor-infiltrating immune cells in  
13 cancers. It is, in great part, shaped by the malignant cells, as in a given cancer type,  
14 tumors presenting different oncogenic processes have different immune contextures.  
15 Moreover, the immune contexture in metastatic sites reflects that of the corresponding  
16 primary tumors. Finally, the components forming the immune contexture represent  
17 targets and markers of efficient anti-cancer immunotherapies.

## 19 **Introduction**

20 Cancers grow and spread in tissues where the malignant cells interact with blood and  
21 lymphatic vessels, stromal cells and hematopoietic cells involved in immune and  
22 inflammatory reactions. This landscape constitutes the immune contexture of a tumor  
23 which is of paramount importance for patients' clinical outcome, is predictive of  
24 responses to therapies and helps identifying novel targets for immunotherapies [1].

## 26 **Clinical impact of the immune contexture in different primary tumors**

28 The immune contexture is a concept that emerged from studies mostly performed in  
29 human colorectal cancer (CRC). A comprehensive analysis of a large collection of  
30 primary CRC tumors revealed that a high density of intratumoral memory T cells  
31 correlates with patients' longer disease-free (DFS) and overall (OS) survivals [2],  
32 confirming a previous report in ovarian cancer [3]. A closer histopathological analysis  
33 of CRC tumors highlighted the fact that the T cells were not stochastically distributed  
34 within the tumor microenvironment but were present in the center (CT) and the  
35 invasive margin (IM) of the tumor nests [4]. The densities of memory CD8<sup>+</sup> T cells in  
36 both the CT and the IM were associated with favorable prognosis, as well as the  
37 expression of genes encoding Th1 cytokines (IFN- $\gamma$ , IL2) and cytotoxic mediators  
38 (granzymes, granzysin)[4]. A comprehensive approach of all genes having any type of  
39 correlation with genes whose expression correlated with both clinical outcome and  
40 tumor infiltration by T cells predicted that certain chemokines (CXCL13, CXCL9,  
41 CXCL10) were involved in shaping an efficient immune microenvironment [5] and  
42 foster T cells activation [6].

43 In parallel to these studies in CRC, our group has investigated the immune  
44 microenvironment of Non-Small-Cell-Lung Cancer (NSCLC). In addition to confirming  
45 the beneficial effect of high densities of T cells with a Th1 orientation and of cytotoxic  
46 CD8<sup>+</sup> T cells, these studies revealed the essential role of tumor-associated Tertiary  
47 Lymphoid Structures (TLS), lymphoid aggregates that structurally resemble secondary  
48 lymphoid organs [7][8]. These formations are present in the invasive margin and in the

49 stroma of lung tumors and absent in distant non-tumoral lung tissue. They are  
50 organized in a T-cell and a B-cell zone surrounded by High Endothelial Venules (HEV).  
51 T cells are in contact with mature DC, and B cells with follicular dendritic cells (FDC)  
52 and tingible-body macrophages. B cells express the activation-induced deaminase  
53 (AID) enzyme necessary for somatic hypermutation and immunoglobulin isotype  
54 switching, a marker of active germinal center (GC) [9]. These data led us to hypothesize  
55 that TLS are sites where immune reactions toward tumor-associated antigens are  
56 generated [10]. Since they are exclusively present within TLS, naïve T and B cells may  
57 have emigrated from peripheral blood *via* HEV. Thus, TLS may represent an immune-  
58 privileged site where naïve lymphocytes may be protected from the  
59 immunosuppressive and inflammatory milieu of the tumor during their differentiation  
60 phase. Indeed, high density of these tumor-associated TLS positively correlates with  
61 NSCLC patient's prognosis [8][9][7]. This is in accordance with the fact that HEV are the  
62 only type of blood vessels which positively correlates with favorable clinical outcome of  
63 cancer [11]. TLS-infiltrating naïve B cells differentiate into memory B cells and plasma  
64 cells which produce anti-tumor antibodies [9]. T cells in TLS are educated by antigen-  
65 presenting DC, become memory lymphocytes and migrate into other tumor areas,  
66 resulting in local control, and to peripheral lymph nodes where they restrain metastatic  
67 spread[10]. TLS likely influence the functional orientation of tumor-infiltrating T cells,  
68 as intratumor T cells have a Th1 and cytotoxic CD8 orientation in tumors having high  
69 TLS densities [8].

70 These initial observations in CRC and NSCLC have been extended to most cancer types  
71 [1][10]. Indeed the evaluation of the immune contexture involves quantitative  
72 immunohistochemistry and gene expression techniques (for evaluation of Th  
73 orientation for instance) and both type of assays needs expertise to be performed  
74 successfully. Regarding T cell infiltration, an "Immunoscore" has been defined  
75 quantifying by immunochemistry two out of the three markers, CD3, CD8 and CD45RO,  
76 in the invasive margin and the center of tumors[12]. A general trend emerges that high  
77 densities of memory T cells with a Th1 orientation and CD8<sup>+</sup> phenotype correlate with  
78 longer DFS and OS also in ovarian, bladder, breast, prostatic, head and neck, and  
79 cervical cancers, hepatocellular carcinoma and melanoma (reviewed in [1] and [13]).

80 Regarding TLS or Ectopic Lymphoid Structures, their quantification has been  
81 performed using the DC-Lamp marker, lymphoid aggregates in HES sections, CD20  
82 positive follicles or PNAd<sup>+</sup> marker for HEV. Whatever the methodology used, the data  
83 show that high densities of TLS also correlate with favorable clinical outcome in CRC,  
84 breast, gastric, pancreatic, colorectal and renal cancers, oral squamous carcinoma,  
85 Merkel cell carcinoma, Warthin tumor as well as melanoma (reviewed in [10]). These  
86 studies establish the immune contexture as a well-studied, robust and clinically  
87 relevant characteristic of human cancers biology.

88 However T and B lymphocytes, and TLS are far from being the only components of the  
89 immune and inflammatory microenvironment of tumors. The densities and  
90 organization of these other cell types also influence patient's clinical outcome although  
91 with less significant power. Indeed the identification of some cell subpopulations such

92 as Treg[14] or MDSC can be hampered by the lack of consensus markers. However, a  
93 trend emerges that high densities of myeloid cells, such as macrophages, MDSCs and  
94 mast cells, and particularly M2 macrophages are associated with poor  
95 prognosis[13][15][16][17][18]. NK cells infiltrating tumors can be anergic[19][20].  
96 Extensive NK cells infiltration has nonetheless usually been associated with favorable  
97 patient outcome[6][21][22][23].

98 Regarding the other T cell subsets, their impact may depend on the cancer type. It is the  
99 case for Th2, T<sub>reg</sub> or Th17 (reviewed in [1]). High densities of DC, T<sub>FH</sub> and B cells in TLS  
100 correlate with favorable prognosis[6]. In presence of low or no TLS, high densities of  
101 stromal B cells, CD8<sup>+</sup> T cells and DC are associated with poor prognosis, and may  
102 support pro-tumoral inflammation and T cell anergy, underlining again the importance  
103 of the location of the immune cells in the tumor landscape [8].

104 Despite the general agreement on the favorable prognostic value of the density of Th1  
105 and CD8<sup>+</sup> T cells, discordant results have been reported in clear cell RCC (ccRCC) [24],  
106 Hodgkin lymphoma [25] or uveal melanoma [26]. Intrigued by these results, we  
107 recently analyzed a cohort of primary ccRCC and confirmed that high densities of CD8<sup>+</sup>  
108 T cells in the IM correlated with shorter DFS and OS [27]. Even more intriguingly, high  
109 expression of the IFN- $\gamma$ , TBX21, and granzyme-B genes were a highly significant marker  
110 of poor prognosis [27]. These results prompted us to study the immune contexture of  
111 ccRCC. We found that there were few TLS in most ccRCC tumors [28][10]. Most CD8<sup>+</sup> T  
112 cells presented an exhausted phenotype with concomitant expression of LAG3 and PD-  
113 1, and tumor cells expressed PD-1 ligands [27]. Patients with tumors containing high  
114 densities of PD-1<sup>+</sup> CD8<sup>+</sup> T cells, and PD-L1 or PD-L2 expression by tumor cells had the  
115 worst prognosis. Strikingly, in most ccRCC tumors, immature DC-Lamp<sup>+</sup> DC were found  
116 outside TLS, close to blood vessels (and not to HEV). It is therefore likely that these DC  
117 instructed incoming T cells in an inflammatory and immunosuppressed milieu  
118 resulting in an abortive immune response. The IFN- $\gamma$  production by T cells has been  
119 reported to induce PD-L1 and/or PD-L2 on tumor cells [29], resulting in an exhaustion  
120 of the immune reaction. Indeed, in the few tumors with high densities of mature DC  
121 inside TLS, high CD8<sup>+</sup> T cells density correlated with favorable prognosis, supporting  
122 the role of TLS in educating HEV-penetrating naive T cells to recognize tumor-  
123 associated antigens and to control cancer aggressiveness [27]. These data suggest that  
124 certain tumor types may be characterized by a disrupted immune contexture, most  
125 likely governed by the malignant cells.

## 127 **Clinical impact of the immune contexture in metastatic sites**

128 It is intuitively thought that tumor progression is accompanied by tumor escape from  
129 the immune system [30][31]. If this escape linearly followed cancer progression, the  
130 immune contexture of metastatic sites should not impact clinical outcome. However,  
131 high densities of CD8<sup>+</sup> T cells in hepatic and lung metastases of CRC correlate with  
132 longer OS [28], as in primary CRC [4]. Densities of infiltrating immune cells were shown  
133 to be correlated between the primary tumors and matched metastases[28]. These

134 findings both suggest that the malignant cells are prominent in shaping their immune  
135 microenvironments. This hypothesis is strengthened by the study of lung metastases of  
136 ccRCC. In contrast to lung metastases of CRC in which high TLS and CD8<sup>+</sup> T cell  
137 densities correlate with favorable prognosis, lung metastases of ccRCC have few TLS,  
138 and high CD8<sup>+</sup> T cell densities correlate with shorter OS, as in primary ccRCC[28]. In  
139 addition, transcriptomic analyses revealed a higher expression of genes involved in  
140 inflammation, immunosuppression and angiogenesis in lung metastases from ccRCC  
141 than in lung metastases from CRC, confirming differences in the functional orientations  
142 of these immune contextures[28].

### 143 **Clinical impact of the immune contexture within a given cancer**

144 Whole genome transcriptomic analyses provide a novel way to classify subgroups in a  
145 given cancer type. These unsupervised approaches complement genomic classifications  
146 by identifying malignant cell subgroups with distinct functional traits. We undertook an  
147 analysis of the expression of immune genes and the concomitant immune cell  
148 infiltration in cohorts of various human cancers. To precisely analyze the immune  
149 contexture of large collections of human cancers, we established transcriptomic  
150 signatures based on the specific expression of genes in a given hematopoietic subset.  
151 We identified robust immune metagenes for lymphocytes (T and B cell subsets, NK, T $\gamma$  $\delta$ ,  
152 cytotoxic cells), myeloid cells (macrophages, monocytes, granulocytes, DC) as well as  
153 endothelial cells and fibroblasts (EB submitted). These signatures were validated *in*  
154 *vitro* in mixtures of hematopoietic and tumor cells and *ex-vivo* on CRC tumor sections.  
155 They were completed by analyses of genes expressed modulating immune functions  
156 (cytokines, chemokines, MHC class I) that sign the functional orientation of the immune  
157 microenvironments. We review herein our data in two cancers, prototypic for the  
158 clinical impacts of their immune contextures, CRC and ccRCC.

159 Different molecular classifications have been proposed for CRC and merged in a four-  
160 subgroups consensus classification [32]. It identifies a microsatellite-instability (MSI)-  
161 enriched group with good prognosis, a mesenchymal subgroup with the worst  
162 prognosis, and KRAS mutated and canonical subgroups with intermediate prognosis.  
163 We applied the immune signatures to these classifications. We analyzed three cohorts  
164 of CRC including over 2000 patients and found that molecular subgroups had distinct  
165 immune signatures. The hypermutated MSI-enriched subgroup had the highest  
166 infiltration of T cells along with cytotoxic lymphocytes, followed by the mesenchymal  
167 subgroup which also presented with high lymphocytic infiltration in the context of high  
168 myeloid cell infiltration as well as extensive presence of endothelial cells and  
169 fibroblasts [33]. The two other groups of tumors had very poor expression of the  
170 immune gene signatures. These analyses show that the malignant cells can influence  
171 the density of immune and inflammatory cells in tumors. The hypermutated MSI-  
172 enriched group had the highest expression of genes involved in T cell chemotaxis  
173 (CXCL9, CXCL10, CXCL16), T cell activation (IFN- $\gamma$ , IL15), T cell inhibition (PD-L1, PD-  
174 L2, CTLA-4, LAG3), and TLS formation (CXCL13). The mesenchymal subgroup had the  
175 highest expression of genes involved in myeloid cell chemotaxis (CCL2), angiogenesis  
176 (VEGF, PDGF), immunosuppression (TGF $\beta$ ), and inflammation (CCL2, complement-

related genes). The two immune low groups had also the lowest MHC Class I expression [33]. Taken together, these data support the concept that the MSI-tumors, which have defects in the DNA-repair enzymes, produce mutated neo-antigens that activate T cells, concurring to shape a favorable immune contexture. In contrast, the mesenchymal tumors, through a high production of angiogenic and inflammatory molecules shape a disrupted immune contexture where inflammation fuels tumor growth while T cells are attracted, but not properly educated towards tumor antigens, resulting in a poor clinical outcome. Finally, tumors from conventional precursors and with high activation of the Wnt pathway, down-regulate their MHC Class I molecules and escape the T cell attacks, which correlates with intermediate prognosis.

We also analyzed the immune signatures in ccRCC primary tumors from metastatic patients treated with Sunitinib, a tyrosine kinase inhibitor. Unsupervised transcriptomic analyses identified four molecular subgroups [34]. Patients of two subgroups responded well to Sunitinib treatment, and consistently had longer Progression-Free Survival (PFS) and OS. The patients of the two other subgroups poorly responded to Sunitinib treatment, and had the worst prognosis. The analyses of the immune signatures revealed that the worst prognostic group had the highest infiltration with lymphoid cells in the context of a high myeloid cell infiltration. Genes involved in T cell chemotaxis (CXCL9, CXCL10, CXCL13), Th1 orientation (IFN- $\gamma$ , IL12, TBX21), T cell exhaustion (PD1, PD-L1, LAG3) and inflammation (TNF- $\alpha$ , CSF1) were highly expressed. The other bad prognosis group had the highest NK cell infiltration and the lowest expression of genes related to adaptive immunity including MHC Class I [32].

Altogether, this analysis of two prototypic tumors showed that within each cancer type, an immunological classification identifies different subtypes of patients with different clinical outcomes corresponding to different oncogenic processes. Thus, we already identified three cancer types (Fig 1):

- the “immunogenic tumors” with production of immunogenic peptides, and an organized immune contexture that generates anti-tumor T cells and antibodies, resulting in a favorable prognosis,
- the “inflammatory tumors” with production of inflammatory, angiogenic and immunosuppressive molecules that disrupt the immune contexture and attract pro-tumor myeloid and lymphoid cells, resulting in exhausted tumor aggressiveness and poor patient’s clinical outcome,
- the “escaping tumors” which down regulate their antigen-presenting machinery produce little or no chemokines and cytokines, and are blind to the immune attack. Patients with such tumors have an intermediate prognosis.

These classifications not only reveal pathological features of cancers but also offer targets and predictive markers for immunotherapies (Table 1).

## **The immune contexture and response to therapy**

217 The immune microenvironment of tumors not only reflects the oncogenic processes of  
218 a cancer in a patient, but is also a constitutive arm of cancer control and thus of  
219 patient's clinical outcome. It is therefore likely that therapeutic interventions modifying  
220 the immune contexture will result in profound changes in cancer evolution.  
221 Characterizing the immune contexture or the corresponding molecular profile of a  
222 tumor in a patient will allow clinicians to propose the most appropriate therapies. For  
223 instance, MSI CRC tumors with high mutational load respond to PD1 axis blockade [35].  
224 Consistent results have been observed in NSCLC [36]. In melanoma patients treated  
225 with anti-PD-1 antibodies, responding tumors are characterized by the entry of pre-  
226 existing CD8<sup>+</sup> T cells from the IM to the CT[37], exemplifying the role of T cell location  
227 into tumors[4]. In cervical carcinoma [38] or pancreatic cancer [39], responses to  
228 therapeutic vaccination are accompanied by the increase of TLS in the tumor vicinity.  
229 In ccRCC patients responding to anti-PD-1 or anti-PD-L1 antibodies, tumors express  
230 both molecules on their infiltrating lymphocytes and tumor cells, respectively [40]. In  
231 bladder cancer, the expression of PD-L1 on infiltrating immune cells was found  
232 essential for response to this immunotherapy [41].

233 For immune silent tumors, bi-specific antibodies direct to tumor antigens may attract  
234 and activate T cells into responding tumors[42]. T cells-based therapies aim also to  
235 bring missing memory T cells into tumors. Strikingly, therapies with antibodies  
236 recognizing tumor-associated antigens, such as CD20 [43], or HER2-Neu [44], are  
237 capable of inducing a memory anti-tumor T cell response responsible for the long-term  
238 effect of these therapies. In tumors where class I MHC molecules are downregulated,  
239 autologous NK cells transfer, recombinant IL15 or KIR-blocking antibodies could foster  
240 NK cells activation and MHC-negative tumor cells elimination[45]. Finally, the immune  
241 microenvironment is implicated in the long lasting effects of chemotherapies and  
242 radiotherapies, by creating an appropriate microenvironment associated with the  
243 release of immunogenic molecules by the tumor cells.



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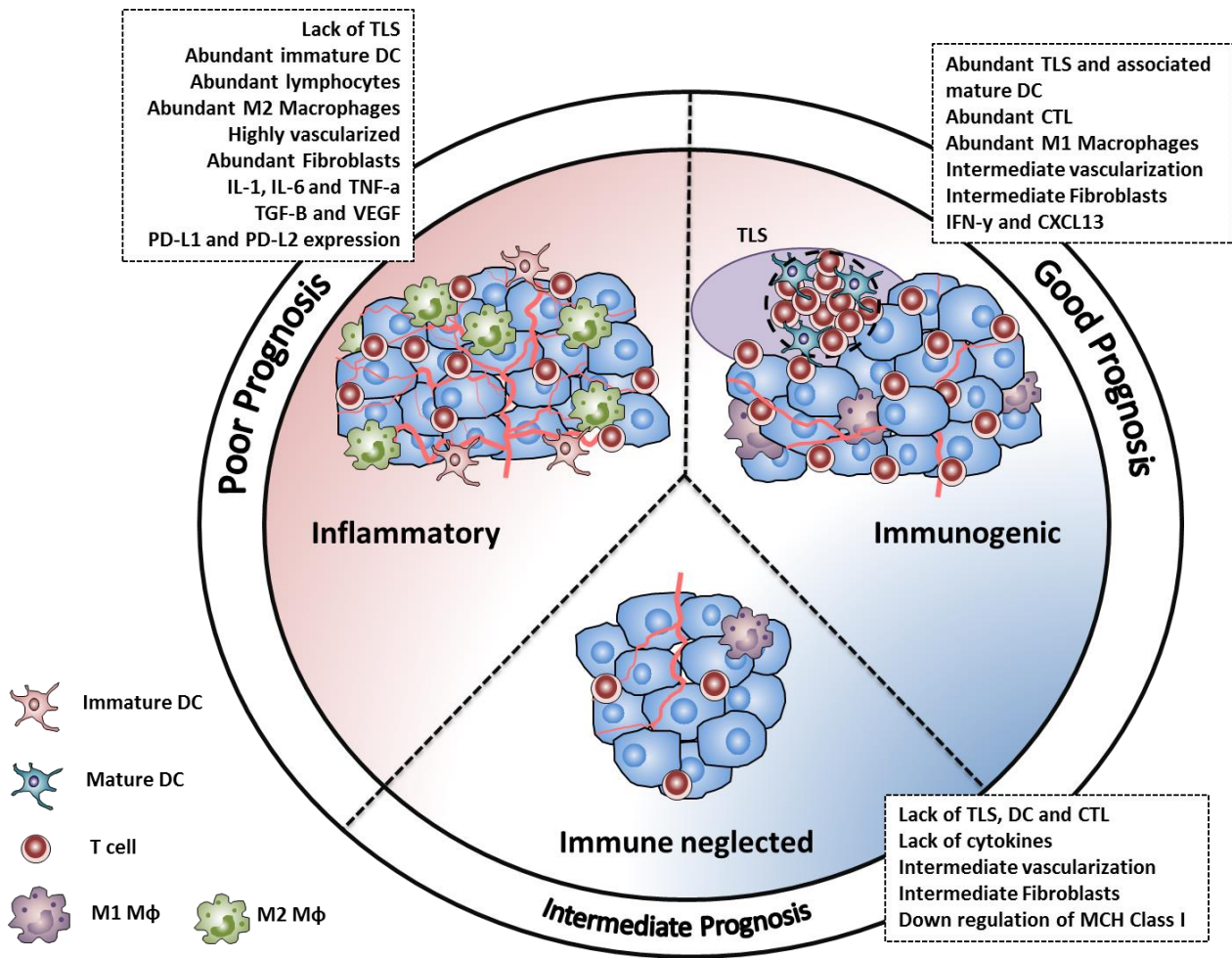
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## References of interest and outstanding interest

- [1] \* A review on the impact of the immune contexture in various cancers
- [3] \* A demonstration that CD3<sup>+</sup> T cells densities correlate with favorable outcome in a human cancer
- [4] \*\* The demonstration of the role of the density, location and functional orientation of T cells in controlling the outcome of a human cancer
- [6] \*\* A comprehensive analysis of the immune contexture in colorectal cancers depicting the clinical impact of all immune cell populations, their locations, functional orientations and their relationship with cancer stage
- [7] \* The first demonstration of the clinical impact of TLS in cancer patients
- [8] \* The demonstration that TLS shape intratumor CD8<sup>+</sup> T cell responses
- [9] \* The demonstration that intratumor B lymphocytes produce anti-tumor antibodies
- [10] \* A comprehensive review of the role of TLS in cancers
- [27] \* The analysis of the impact of components of the immune contexture in the shaping an efficient or deleterious anti-tumor immune reaction
- [28] \* A demonstration of the clinical impact of the immune contexture in metastatic cancer sites
- [35] \*\* A clinical trial demonstrating that inhibition of immune checkpoint results in longer survival in colorectal-cancer patients with hypermutated MSI tumors



421  
422 **Figure 1.**

423 **The immunological wheel.** Cartoon depicting the three immune contexts that can be induced in tumors. The 'immunogenic tumors' are characterized by abundant Cytotoxic T-Lymphocyte (CTL) infiltration, the presence of Tertiary Lymphoid Structures (TLS) and low/moderate vascularization while associated with the longest patient's survival. The 'immune neglected' tumors are characterized by lack of infiltration by immune cells, low/moderate vascularization and intermediate prognosis. Finally, the 'inflammatory tumors' are characterized by abundant CTL in the absence of TLS, conspicuous infiltration with M2 macrophages, severe vascularization and poor prognosis.

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Immune subgroup	Examples of corresponding molecular subgroups	Microenvironment characteristics	Escape mechanisms	Immunotherapeutic goals	Potential immunotherapeutic approach
<b>Immunogenic</b>	CRC Hypermutated	High T cell infiltration with Th1 orientation and cytotoxic lymphocytes	Immune checkpoints : PD1 axis, LAG3, CTLA4	Boost intratumor cytotoxic T cells	Checkpoint-blockade
<b>Inflammatory</b>	CRC Mesenchymal  ccrcc4	High lymphocyte and myeloid cells infiltration High angiogenesis Stromal mesenchymal cells	Hypoxia TGFβ PD1 axis	Dampen inflammation and associated suppressive mechanisms Establish normoxia Boost intratumor suppressed cytotoxic T cells	Anti-angiogenic Anti TGFβ Checkpoint-blockade
<b>Immune neglected</b>	CRC Canonical and Metabolic  ccrcc1 and ccrcc2	Low lymphocytic and myeloid cells infiltration	Low class I MHC expression	Attract cytotoxic T cells in tumors Bypass class I MHC presentation	CAR T cells Bi-specific antibodies

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**Table 1.**

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**Immunotherapeutic approaches tailored for tumor immune subgroups.**

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The three immune subgroups, their hallmarks, immune escape mechanisms are listed, as well as the corresponding immunotherapeutic goals and potential approaches.

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CRC : Colorectal cancer

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ccrcc : clear-cell Renal Cell Carcinoma

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