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Cross-talk between cancer-initiating cells and immune cells: considerations for combination therapies

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DEMO Cancer initiating cells (CIC) or cancer stem-like cells (CSC) represent a small, distinct population of cancer cells best characterized by their high tumorigenicity. They undergo asymmetric cell division that results in repopulation of the bulk of the tumor and self-renewal. CICs are likely responsible for treatment failure and tumor recurrence, as they are highly resistant to traditional therapies, such as chemo- and radiotherapy (1). This resistance is partly due to their proliferative quiescence and increased anti-apoptotic features. Immune targeting is an emerging alternative approach, which may override these resistance mechanisms. However, relatively little is known about the interaction between CICs and immune cells that have the capacity to recognise and destroy not only the bulk of the tumor but also CICs.

In squamous cell carcinoma of the head and neck (SCCHN), CICs have been identified as a subpopulation of CD44+ cells. In a recent publication (2), Lee *et al.* carried out a comparative study between the immune behaviour of CD44+ and CD44- cells in SCCHN. The CD44 marker alone is not sufficient to define the CIC population without other markers. ALDH bright cells e.g., represent a subpopulation of CD44+ cells displaying enhanced clonogenic, tumorigenic capacity and radioresistance (3). Nevertheless, the study (2) provides some novel information about potential enhanced immunosuppressive features of the CIC-containing population of tumor cells. The key observation is the preferential expression of the immune checkpoint molecule programmed death-ligand 1 (PD-L1) on CD44+ vs. CD44- SCCHN cells. PD-L1 binds to PD-1 on T cells, inhibiting their function. In this study, IFN γ secretion by autologous CD8+ tumor-infiltrating lymphocytes

(TIL) was inhibited by CD44+ cells. In another study, using a SCCHN cell line, CD44+ cells have also been shown to produce more TGF β and IL-8, but not TNF α and IL-6, compared to that by CD44- cells (4). These CD44+ cells also inhibited T cell proliferation more strongly than CD44- tumor cells. However, neither study addressed the question of how susceptible these tumor cells are to T cell killing. Highly differentiated cytotoxic T lymphocytes (CTL) can be fairly resistant to immunosuppression, thus the effect of CD44+ tumor cells on CTL effector function would have been a more relevant T cell function to study. Indeed, it has been shown that SCCHN CICs, grown in 3D spheroids and identified by elevated ALDH levels, Nanog, Sox and Oct3/4 expression, are more susceptible to allo-specific T cell killing than tumor cells that are lower or negative for these markers (5).

Successful targeting of CICs by antigen-specific T cells in other types of cancer has also been demonstrated. Novel/mutated, overexpressed, oncofoetal, post-translationally altered and cancer testis (CT) are types of tumor-associated antigens T cells recognize (6). In the SCCHN study, discussed above (2), the cognate antigens were not identified so the question remains whether the proportions of tumor cells were comparable in the CD44+ and CD44- populations and if they differed in their antigen profile. As antibody blockade of the PD-1/PD-L1 interaction only partially reversed the inhibitory effect on autologous TIL-derived T cells, this possibility cannot be excluded. Biomarkers of CICs (e.g., ALDH1 or CD133) are also being investigated as immune targets (7). ALDH1A1-specific CD8+ T cells were shown to kill ALDH (bright) SCCHN CICs *in vitro* and T cell transfer resulted in better tumor control *in vivo* (8). However, the variety and reliability of

63 markers associated with CICs, along with the potential
 DEMO 64 for damaging healthy stem cells, expressing stem-related
 65 markers, may detract from this approach. CT antigens have
 66 been found to be frequently expressed in SCCHN (9), with
 67 e.g., MAGE A3/6 being an independent prognostic factor
 68 for tumor recurrence. Thus, CTL or monoclonal antibodies
 69 targeting CT antigens may be effective against both CICs
 70 and non-CICs in SCCHN. Interestingly, some antigens
 71 specific to CICs (e.g., BORIS, DNAJB8) have been reported
 72 to be more immunogenic than “shared” antigens (10,11). On
 73 the other hand, CD271+ melanoma CICs frequently lack
 74 the expression of melanoma tumor antigens TYR, MART1
 75 and MAGE C1/C2 (12). This means that targeting these
 76 antigens would not affect CIC survival.

77 The antigen presentation ability of CICs, enabling
 78 interactions between cytotoxic T cells and peptide-HLA/
 79 MHC complexes, is also under debate. Low HLA class I
 80 expression and defective antigen processing machinery have
 81 previously been reported in CICs, in common with healthy
 82 stem cells (13). Contrastingly, Lee *et al.* reported that
 83 CD44+ and CD44- cells expressed comparable, high levels
 84 of HLA-ABC in the presence or absence of IFN γ (2). This
 85 suggests the differences in T cell modulation by CD44+
 86 and CD44- cells may not be associated with their antigen
 87 processing ability. It may be useful to extend this line of
 88 investigation by e.g., transfecting CD44+ and CD44- cells
 89 with a surrogate antigen and determine the cognate T cell
 90 response.

91 A successful adaptive immune response, in which effector
 92 T cells eliminate CICs in an overt tumor and memory T
 93 cells persist to eliminate emerging CICs, would be crucial to
 94 achieve long-term tumor control. However, the interaction
 95 between tumor-infiltrating immune cells and CICs is still
 96 poorly understood as most studies are conducted *in vitro*
 97 rather than *in situ*. Whilst numerous immunosuppressive
 98 mechanisms have been attributed to CICs (14), most of
 99 these mechanisms are not unique to this population of
 100 cancer cells. There is a possibility that CICs are immune-
 101 protected rather than being immunosuppressive in their
 102 own right. CICs reside within cellular ‘niches’, which aid
 103 their immune protection and survival. These may include
 104 enhanced chemoattraction of tumor-associated macrophages
 105 (TAM) by CICs and skewing towards immunosuppressive
 106 M2-type macrophages (15,16). In SCCHN, self-renewal
 107 of CICs, identified by CD44 and ALDH expression, has
 108 been promoted by endothelial cell-secreted factors (17).
 109 Furthermore, 80% of these CICs were found in close
 proximity to blood vessels in the tumor tissue. Elimination

110 of tumor-associated endothelial cells significantly reduces
 111 the proportion of CICs in xenografts (17). These findings
 112 indicate that endothelial cell-initiated signaling can enhance
 113 the survival and self-renewal of CICs, providing a unique,
 114 protective microenvironment for these cells.

115 The observation that PD-L1 is expressed on some
 116 CD44+ cells (but not on CD44- cells) in SCCHN (2)
 117 seems to confirm the theory that CICs are associated with
 DEMO 118 enhanced immune protection. PD-1/PD-L1 inhibition as a
 119 therapeutic target in SCCHN is being tested in numerous
 120 clinical trials both alone and in combination (18). PD-L1
 121 can also be expressed on infiltrating immune cells, as an
 122 effect of e.g. stromal factors, as shown by Spary *et al.* (19).
 123 However, correlation between PD-L1 expression on
 124 tumor cells or immune infiltrates and response to therapy
 125 has not been confirmed (20). Nevertheless, PD-L1
 126 targeting maybe crucial for releasing the full potential of
 127 tumor antigen-specific effector T cells to target not only
 128 PD-L1+ differentiated tumor cells but also CICs and
 immunosuppressive TAMs.

129 As a conclusion, further studies with more precise
 130 identification of CICs and better definition of both their
 131 immunosuppressive nature and susceptibility to immune
 132 attack are clearly needed. Future treatment combinations
 133 may be improved by simultaneous targeting of the
 134 microenvironment, immune checkpoints, CICs and the
 135 bulk of the tumor in order to deliver direct and indirect hits
 136 both for tumor destruction and protection from recurrence.

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Footnote

139 *Provenance:* This is a Guest Commentary commissioned by
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143 *Conflicts of Interest:* The authors have no conflicts of interest
 144 to declare.

145 *Comment on:* Lee Y, Shin JH, Longmire M, *et al.* CD44+
 146 Cells in Head and Neck Squamous Cell Carcinoma
 147 Suppress T-Cell-Mediated Immunity by Selective
 148 Constitutive and Inducible Expression of PD-L1. Clin
 149 Cancer Res 2016;22:3571-81.

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