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Immunotargeting of tumor subpopulations in melanoma patients

A paradigm shift in therapy approaches

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Abbreviations: NED, no evidence of disease; OS, overall survival; RF, recurrence-free; TIBC, tumor- infiltrating B cell; TME, tumor microenvironment

Several melanoma cell subpopulations with tumor-initiating and/or tumor-maintaining properties exist that may contribute to chemoresistance and tumor recurrence after standard therapies. One of these subpopulations expresses a B-cell marker, CD20. In a small pilot trial, we showed that a subset of Stage IV melanoma patients may potentially benefit from an adjuvant treatment using the anti-CD20 antibody rituximab.

Despite the recent therapeutic breakthroughs, melanoma is not curable when it has spread to distant sites. Melanomas are heterogeneous tumors, comprised of several genotypic and phenotypic subtypes. Given this complexity, therapeutic approaches designed to treat melanomas as a single disease using conventional chemotherapy or targeted agents all bear the risk to eventually promote resistance.[1] This may be particularly true for slow cycling or non-proliferating tumor cells. These cells may contain tumor-initiating subpopulations that drive tumor progression through cycles of quiescence, self-renewal and differentiation, a capacity that may be regulated by distinct signals from the tumor microenvironment (TME). In melanoma, several cell subpopulations with tumor-initiating and/or tumor-maintaining properties have been described (such as melanoma subpopulations expressing CD20, CD133, CD271, ABCB5 and/or JARID1B)[1] and there is a growing evidence that these small and transient subpopulations are induced by TME-derived factors that are directly influenced by chemotherapy, radiation therapy or host immunity.[1]

In 2005, Fang et al. were the first to describe a small subpopulation of melanoma cells expressing the B-cell surface marker CD20 when they were grown as tumor spheroids.[2] Such a CD20$^+$ population was indicated as "cancer stem cell-like" or "tumor-initiating" as these cells fulfilled the criteria of "tumor stemness" by their ability to differentiate into multiple different cell lineages and being more tumorigenic than the CD20-negative population in a preclinical xenotransplantation model. In the meanwhile, we and others have identified CD20-expressing melanoma cells in metastatic lesions from melanoma patients.[3] Interestingly, gene expression profiling identified CD20 as one of the top 22 genes in melanoma defining the aggressive nature of the disease,[4] with expression levels increasing along disease progression (unpublished data). Our unpublished in vitro observations also indicate that melanoma cells that are resistant to the chemotherapeutic drug cisplatin exhibit an enhanced expression of CD20.

Based on the these observations, we hypothesized that melanoma patients at high risk for disease recurrence could benefit from an adjuvant treatment specifically targeting the CD20$^+$ melanoma cell subpopulation (**Fig. 1**) and conducted a clinical pilot trial in a small cohort of advanced stage melanoma patients (clinical Stage IV) who had been rendered disease-free by standard therapies.[3] Nine patients received the anti-CD20 antibody (rituximab) for 2 years or until disease recurrence. Even though therapy was stopped after 2 year, six out of nine patients were still alive after a median observation of 42 monts and five of them were recurrence-free (RF). Interestingly, before the adjuvant anti-CD20 antibody therapy, eight of such nine patients experienced one to four complete remissions after standard therapy, in each case followed by tumor recurrence. Consistent with published data, the median of these RF-intervals following standard therapies was 6 months, while the median of RF-intervals following anti-CD20 treatment was 42+ months, in the very same patients.[3]

Recently, Schmidt et al. have observed in a preclinical xenograft model complete inhibition of growth and recurrence of highly tumorigenic human melanoma cells following the administration of autologous T cells genetically engineered to express a chimeric CD3ζ/CD20 antigen receptor, which specifically target CD20$^+$ cells.

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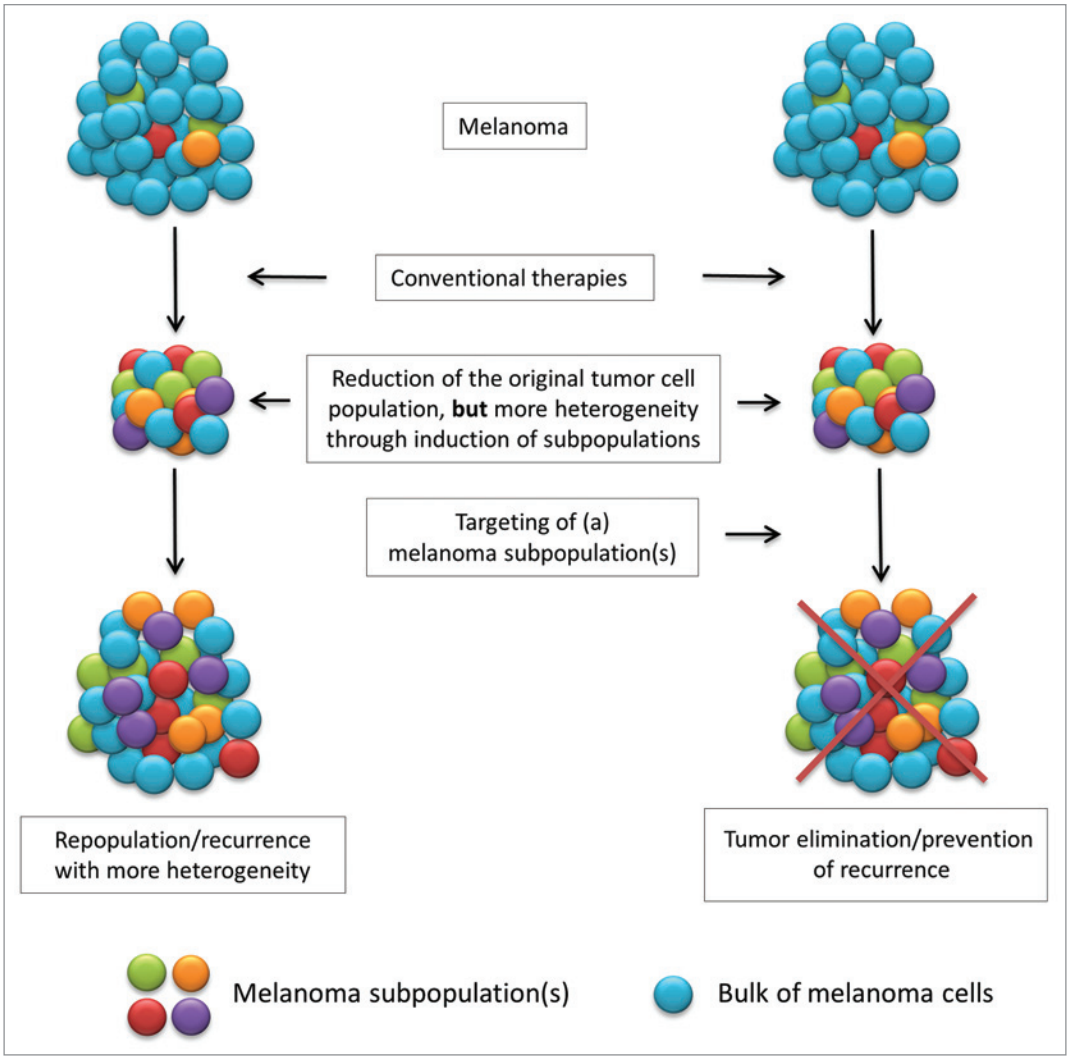

Figure 1. Immunotherapy targeting (a) melanoma cell subpopulation(s). Melanoma contains several distinct cell subpopulations with tumor-initiating and/or tumor-maintaining capacity. These may be resistant to or activated by conventional therapy leading to a more heterogeneous tumor, even though the bulk of the original tumor cell population may have responded to treatment. Chemoresistant and/or therapy-induced tumor cell subpopulations may then have the capacity to repopulate the tumor. Selective targeting of these melanoma cell subpopulations, either in an adjuvant or neoadjuvant setting, may prevent tumor repopulation and recurrence of the disease.

Inhibition of tumor growth was long-lasting and the RF-interval in mice was longer than 36 weeks.[5] Furthermore, in a case report study, Schlaak et al. described the regression of metastatic melanoma lesions upon direct intratumoral administration of an anti-CD20 antibody in a patient undergoing systemic chemotherapy.[6]

As expected, we observed that the anti-CD20 treatment depleted B lymphocytes in the peripheral blood of our patients. B cells are the central component of the humoral immune system and, in the context of cancer, can be important contributors to tumor initiation and growth, as exemplified in the K14-HPV16 transgenic mouse model of inflammation-associated epithelial carcinogenesis.[7] In this model, combined B- and T-lymphocyte deficiency resulted in a failure to initiate and/or sustain leukocyte infiltration and subsequently in significant reduction of carcinoma incidence. Adoptive transfer of B lymphocytes reconstituted chronic inflammation through IgG-mediated stimulation of activating FcγR on resident and recruited myeloid cells.[7] This is particularly interesting, as serological cloning methods showed that most cancer and melanoma patients mount tumor-specific autoantibody responses. Yet another mechanism of tumor promotion is cytokine secretion by tumor-infiltrating B cells (TIBCs). TIBCs have been described in several types of cancers and $CD20^+$ B cells are known to frequently infiltrate melanomas.[3] Through the secretion of interleukin-10 (IL-10) and transforming growth factor β1 (TGF-β1) TIBCs can negatively regulate immune responses, behaving as "so-called" regulatory B cells. TIBCs are also an important source of tumor-promoting cytokines, as recently shown in a mouse model of castration-resistant prostate cancer.[8] In this model, B-cell deficiency or depletion with an anti-CD20 antibody delayed tumor growth, whereas adoptively transferred B cells,

but not T cells, restored tumor growth. These B cells migrated into the tumor and activated IKKα and STAT3 signaling in cancer cells via the secretion of cytokines including lymphotoxin(α:β), an established promoter of melanoma growth.

To conclude, in addition to targeting the bulk of cancer cells, specific targeting of (a) minor cancer cell subpopulation(s) may be required to definitely eradicate melanoma and presumably prevent recurrence of the disease (**Fig. 1**). Combination therapies may use antibodies or inhibitors that directly target chemoresistant subpopulations and/or tumor-promoting subpopulations of the TME and/or neutralize tumor-promoting cytokines present in the TME. Recent reports show that melanoma cell subpopulations can indeed be targeted by different immunotherapeutic approaches leading to reduced tumor growth in preclinical in vivo models.[5,9,10] Though the exact mechanisms underlying the efficacy of anti-CD20 therapy in melanoma patients remain to be defined, the possibility to simultaneously deplete chemoresistant melanoma cell subpopulations and tumor promoting TIBCs may be a unique and particularly attractive feature of CD20 immunotargeting.

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