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EDITORIAL

STAT3 inhibition for cancer therapy: Cell-autonomous effects only?

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ABSTRACT

A paper recently published in *Science Translational Medicine* describes a next-generation antisense oligonucleotide that specifically downregulates the expression of human signal transducer and activator of transcription 3 (STAT3). Such an oligonucleotide, AZD9150, exerts antineoplastic effects on a selected panel of STAT3-dependent human cancer cells growing *in vitro* and *in vivo* (as xenografts in immunodeficient mice). Moreover, preliminary data from a Phase I clinical trial indicate that AZD9150 may cause partial tumor regression in patients with chemorefractory lymphoma and non-small cell lung carcinoma. STAT3 not only participates in cell-autonomous processes that are required for the survival and growth of malignant cells, but also limits their ability to elicit anticancer immune responses. Moreover, STAT3 contribute to the establishment of an immunosuppressive tumor microenvironment. Thus, the inhibition of STAT3 may promote immunosurveillance by a dual mechanism: (1) it may increase the immunogenicity of cancer cells via cell-autonomous pathways; and (2) it may favor the reprogramming of the tumor microenvironment toward an immunostimulatory state. It will therefore be important to explore whether immunological biomarkers predict the efficacy of AZD9150 in the clinic. This may ameliorate patient stratification and it may pave the way for rational combination therapies involving classical chemotherapeutics with immunostimulatory effects, AZD9150 and immunotherapeutic agents such as checkpoint blockers.

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A report from Hong and colleagues recently published in *Science Translational Medicine* provides major advances in our understanding of the role of the transcription factor STAT3 in cancer cell biology, at several levels. First, the paper describes for the first time an antisense oligonucleotide specific for human STAT3 bearing chemical modifications that allow it to freely cross the plasma membrane, and hence to abolish STAT3 expression in cultured human cells at nanomolar concentrations. This oligonucleotide, AZD9150, also reduces STAT3 levels in xenotransplanted human tumors growing on immunodeficient mice.[1] Second, the authors provide convincing evidence that AZD9150 can reduce the proliferation of selected STAT3-dependent cancer cells, most likely via an on-target effect. Such an antineoplastic activity was observed *in vitro*, on human cancer cell lines and freshly isolated primary malignant cells, as well as *in vivo*, on human cancer xenografts implanted into immunodeficient mice.[1] Third, the article reports preliminary data from a Phase I clinical trial aimed at investigating safety profile of AZD9150 in cancer patients. This study identified thrombocytopenia as the main dose-limiting toxicity of AZD9150 (presumably also an on-target effect). Moreover, the authors monitored the circulating levels of interleukin-6 (IL-6), which is a well-characterized transcriptional target of STAT3, as a biomarker of AZD9150 activity, finding that IL-6 was reduced in the circulation of a majority of AZD9150-treated individuals. Finally, several patients with chemorefractory malignancies enrolled in the study (in particular: four subjects with lymphoma and one individual with non-small cell lung carcinoma) achieved partial responses on AZD9150, as monitored on ^{18}F-fluorodeoxyglucose (^{18}F-FDG) positron emission tomography (PET).[1] Unfortunately, no information on the expression of STAT3 in cancer patients before and after the administration of AZD9150 is available.

STAT3 is well known as a transcription factor activated by Janus kinase 1 (JAK1) and JAK2, correlating with the ability of phosphorylated (but not dephosphorylated) STAT3 to translocate from the cytoplasm to the nucleus. Nuclear STAT3 transactivates numerous genes, hence switching on a genetic program that sustains cellular survival, proliferation, as well as the secretion of pro-inflammatory factors that may promote tumor progression.[2-4] However, like several other transcription factors, STAT3 also has cytoplasmic functions.[5] Notably, cytoplasmic STAT3 regulates oxidative phosphorylation by mitochondria and inhibits autophagy, implying that the JAK1- or JAK2-dependent translocation of STAT3 to the nucleus has an immediate effect on cellular metabolism (which manifests with an increase in autophagic flux).[6-9] Based on these considerations, it will be important to study how AZD9150 affects tumor

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metabolism, especially in relationship with ^{18}F-FDG PET.[1] In particular, does AZD9150 truly reduce tumor mass, or does it merely (and perhaps transiently) affect ^{18}F-FDG uptake by cancer cells?

STAT3 inhibition most likely does not exert antineoplastic effects by purely cell-autonomous mechanisms.[4] Indeed, the inhibition of STAT3 in cancer cells is expected to limit the production of pro-inflammatory factors (such as IL-6), hence reducing local inflammatory reactions that may contribute to tumor progression.[4,10-12] Moreover, the inhibition of STAT3 allows malignant cells to secrete high amounts of Type I interferon and other products of so-called interferon response genes (IRGs), including chemokine (C-X-C motif) ligand 9 (CXCL9) and CXCL10.[13,14] By virtue of this mechanism, STAT3 inhibition stimulates the recruitment of immune effectors into the tumor bed and improves immunosurveillance, especially in the context of ongoing anticancer immune responses.[13] Indeed, STAT3 inhibitors can be advantageously combined with immunogenic cell death (ICD)-inducing chemotherapeutic agents.[13] Given the clinical impact of ICD inducers,[15-22] it will be important to explore this possibility in properly designed trials.

STAT3 inhibition may also affect non-malignant compartments of the tumor microenvironment. AZD9150 specifically target human (not murine) STAT3,[1] meaning that its effects on xenografted human tumors evolving in immunodeficient mice must reflect cancer cell-autonomous effects. However, in some of such experiments, AZD9150 only became active against human lymphomas when it was combined with another antisense oligonucleotide that target mouse STAT3 as well,[1] underscoring the contribution of host STAT3 to tumor progression. It is not clear which non-malignant components of the tumor mass (fibroblasts, endothelial cells, tissue-resident macrophages, etc.) contribute to the growth of human tumor xenografts in this particular case. Surely, such an effect cannot be attributed to lymphocytes, as the mice used in these experiments (NOD.Cg-*Prkdc*^{scid} *Il2rg*^{tm1Wjl}/SzJ mice) are severely immunocompromised as they completely lack T and B lymphocytes as well as natural killer (NK) cells.[1]

Experiments performed in immunocompetent mice bearing histocompatible tumors demonstrated that STAT3 also plays major roles in the subversion of anticancer immunosurveillance. For instance, the immunosuppressive functions of myeloid-derived suppressor cells (MDSCs) critically rely on STAT3.[23,24] Mice specifically lacking STAT3 in myeloid cells are indeed more resistant than their wild-type counterparts to carcinogen-induced oncogenesis, and exhibit a superior immunological control of transplanted tumors.[25] Notably, the expression of CD274 (a potent immunosuppressive molecule also known as PD-L1) by MDSCs and other immunosuppressive myeloid cells also depends on STAT3.[26] This latter observation suggests that STAT3 inhibition may downregulate PD-L1, implying that combining AZD9150 with checkpoint blockers[27,28] targeting the interaction between PD-L1 and its main receptor (programmed cell death 1, PDCD1, best known as PD-1) may not be useful. However, this conjecture remains to be investigated at the experimental level.

Undoubtedly, the paper by Hong *et al.* will spur renewed interest in STAT3 as a target for cancer therapy. Given the importance of STAT3 in pro-inflammatory and immunosuppressive pathways, the possibility of using STAT3 inhibitors as immunostimulatory agents (and de facto checkpoint blockers) awaits urgent verification in clinical settings. Moreover, the observations presented above suggest that multiple immunological parameters should be evaluated as possible biomarkers that predict the clinical efficacy of STAT3 inhibition (and hence allow for patient stratification). Finally, the possibility to associate AZD9150 (or its derivatives) with other immunologically active compounds (including, but not limited to, ICD inducers) should be actively investigated.

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

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