Also supporting this is the fact that the addition of exogenous estradiol to IMiD-treated megakaryocytes in culture almost completely restored the number of proplatelet-producing megakaryocytes to that of untreated controls. Furthermore, expression of aromatase, an enzyme that mediates estradiol synthesis, was significantly reduced in CD34$^+$-derived human megakaryocyte cultures 24 hours after treatment with IMiDs. Co-immunoprecipitation of lysates of aromatase and cereblon (the direct target of IMiDs) from K562 cells showed that cereblon appeared with aromatase only when lenalidomide was present. Co-immunoprecipitations with a mutant cereblon that was missing the IMiD binding region failed to show binding, as expected. Bone marrow samples of MM patients receiving IMiDs as part of their treatment were compared with those from patients who were not receiving IMiDs. Remarkably, those patients with IMiD-induced thrombocytopenia did not have detectable levels of aromatase within the bone marrow or within isolated megakaryocytes. However, patients not treated with IMiDs had normal levels of aromatase in their bone marrow.

What about the future? The authors have already highlighted the possibility of administering estradiol in the clinic with its implications for thrombosis and post-menopausal breast cancer. The sensible approach would be to understand on a structural basis why the IMiD-cereblon structures are binding to aromatase and then use this knowledge to identify IMiDs that do not facilitate cereblon and aromatase binding. These findings also raise new questions regarding proplatelet formation. It can be appreciated that pro-platelets are not typically formed in static conditions in vivo. Instead, they are released by megakaryocytes that are packed in a dense marrow environment and are released with shear forces. It would be interesting to investigate the roles and the importance of aromatase and estradiol in proplatelet formation under physiological shear forces using platelet bioreactors. Is it possible to add increasing concentrations of exogenous estradiol and generate more (pro)platelets? If so, would the platelets that are produced function normally? Would we also be able to inhibit estradiol synthesis and learn about the downstream effects, if any, this inhibition could have on the cytoskeleton arrangement? Tochigi and colleagues have done an excellent job combining their ex vivo and mechanistic data to provide a strong argument that IMiDs cause thrombocytopenia through inhibition of proplatelet formation.

Conflict-of-interest disclosure: J.E.I. is a founder of and has financial interest in Platelet BioGenesis, a company that aims to produce donor-independent human platelets from human-induced pluripotent stem cells at scale. N.L.A. declares no competing financial interests. ■

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MYELOID NEOPLASIA

Comment on Joshi et al, page 2159

# ON TRacK towards novel targets in leukemia

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In this issue of *Blood*, Joshi et al report and characterize novel and recurrent NTRK2/3 point mutations in ~5% of patients with different hematologic neoplasms, including acute myeloid leukemia and lymphoblastic leukemia, as well as myeloproliferative disorders.[1]

Molecular testing has evolved into an essential cornerstone in the diagnostic management of patients with both solid and hematologic malignancies. But how large should our testing panels be? Should we limit ourselves to target panels that include known driver genes that are associated with the disease? Should we include additional genes in cancer biology? Do we really need to analyze the entire coding sequence? There is always the hope of identifying unusual driver mutations that can be targeted with an inhibitor. But that hope stands against several variants of unknown significance that have no therapeutic consequence. Thus, logic and streamlined examples are needed to inform our approach to such data so that we can move from our large data collections toward actionable procedures that truly benefit our patients.

In their elegant experimental approach, Joshi et al demonstrated the oncogenicity of 4 of the 9 point mutations under consideration. They characterized the likely underlying patho-mechanism of those mutations, which led to increased cell surface abundance and receptor dimerization which, in turn, caused increased downstream pathway signaling (see figure). All 4 point mutations could successfully be treated with US Food and Drug Administration–approved NTRK
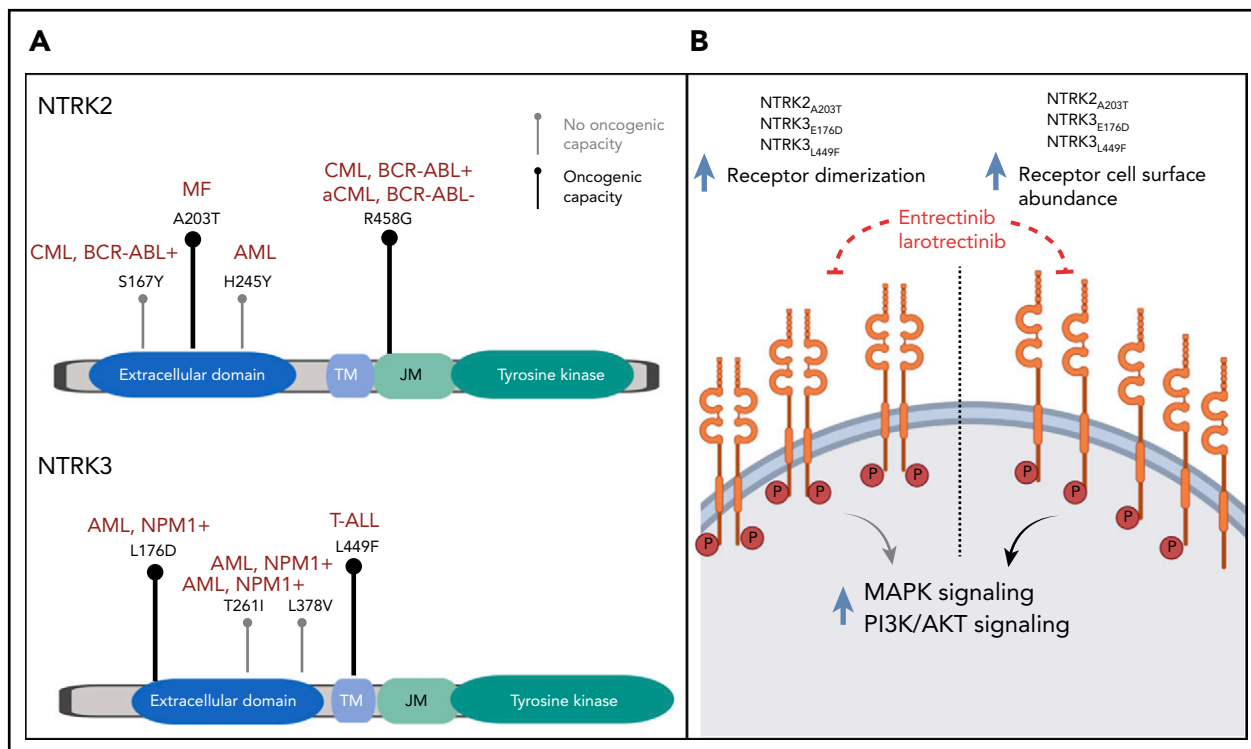
(A) Lollipop plots depicting the identified point mutations in *NTRK2* (upper panel) and *NTRK3* (lower panel) genes. Bold black marks indicate experimentally validated oncogenicity of the mutation; gray marks indicate failure to demonstrate oncogenicity. The diseases of patients in which the point mutations were identified are indicated in dark red. (B) Proposed mechanism by which the identified NTRK mutations increase oncogenicity. AML, acute myeloid leukemia; CML, chronic myeloid leukemia; MF, myelofibrosis; T-ALL, T-cell acute lymphoblastic leukemia.

inhibitors, and importantly, their antileukemic potential was effective even in the presence of mutations in other known driver oncogenes.

The identification and functional characterization as well as proof of targetability of the discovered *NTRK* point mutations is a highly important finding by itself. In contrast to solid tumors,[2,3] the emphasis in hematologic malignancies has mainly been on NTRK fusion genes,[4,5] which have been found to be responsive to NRTK inhibition.[6-9] However, rare examples of somatic point variants warranted a study such as that by Joshi et al which had the goal of analyzing the frequency and relevance of *NTRK* point mutations.[4,10] Given the observed frequency and especially the encouraging targetability of NTRK genes, adding those genes to targeted sequencing panels for hematologic malignancies should now be strongly considered.

The Joshi et al study has an impact that goes beyond that of the described NTRK genes, because the study reinforces general challenges and provides answers to recurring questions in the interpretation of variants detected in sequencing studies.

First, not all detected missense variants within a gene matter, and functional characterization is necessary to determine their relevance. Joshi et al found 9 different variants in 2 *NTRK* genes, but they showed that only 4 of the variants actually had oncogenic potential. Of note, some of these variants were located in highly conserved residues with predicted structure alterations via 3D modeling. This is not a novel concept, but it serves as a reminder that, even though validation experiments take time and effort, they are required in addition to commonly used in silico predictions.

Second, novel mutations that occur in the presence of a known driver mutation may substantially alter disease biology and may represent an important target lesion. In the patients with detected pathogenic *NTRK* mutations, at least 2 of them harbored BCR-ABL and *CSF3R* disease-defining mutations. This seems especially noteworthy for a disease like chronic myeloid leukemia; in the relapse setting, the focus is on detecting known resistance mutations and does not routinely include a broader screening approach.

So large-scale genomic profiling is indeed tedious, often yields results only in mutations of known foes, and is very expensive. But Joshi et al provide a very good example of why, if done the right way, this approach is necessary not only for personalized patient care but also to enhance our understanding of disease biology.

Conflict-of-interest disclosure: The author declares no competing financial interests. ∎

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