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Combining targeted therapy with immunotherapy (interferon-α)

Rational, efficacy in gastrointestinal stromal tumor model and implications in other malignancies

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Abbreviations: IM, imatinib; GIST, gastrointestinal stromal tumor; IFN-α, interferon-α; CR, complete response; PR, partial response; PFS, progression-free-survival; mAb, monoclonal antibody

Imatinib revolutionized gastrointestinal stromal tumor (GIST) treatment but median-progression-free-survival of unresectable/metastatic disease is < 2 y. B-RAF^{V600}-mutated-melanoma responds to vemurafenib dramatically but median-progression-free-survival is < 9 mo. Combining imatinib with immunotherapy (peginterferon α-2b) in GIST showed significant induction of antitumor immunity and highly promising clinical outcomes. This strategy warrants further testing in other malignancies.

Imatinib (IM, Gleevec®, Glivec®),[1] a selective inhibitor of ABL, KIT and PDGFRA/B, represents the first paradigm-shift targeted therapy for gastrointestinal stromal tumor (GIST). Unfortunately, the median-progression-free-survival (PFS) of unresectable locally advanced and metastatic GIST is < 2 y.[2] Vemurafenib (PLX4032/RG7204) demonstrated response rate of 52%, and represents a similar paradigm-shift treatment for B-RAF^{V600}-mutated-melanoma, however, the short median-PFS of < 9 mo is disappointing.[3] Common drug-resistant mechanisms include: (1) Mutation of target evading drug binding, (2) Upregulation of up- or down-stream signaling molecule(s), (3) Alternative pathway(s) bypassing target. The first mechanism dominates IM-resistance in GIST with missense *KIT* mutations commonly located in KIT kinase domain[4] and activation loop. A single amino acid replacement (gatekeeper mutations) is sufficient to alter hydrogen bonds, or hydrophobic support, thus impede drug binding (**Fig. 1A-C**). Although gatekeeper mutations in B-RAF can confer vemurafenib-resistance in laboratory models, so far vemurafenib-resistance in melanoma manifested mainly receptor-tyrosine kinase or N-RAS upregulation, or signaling pathway switch to PDFGRB overexpression.[5]

Drug-resistant clones evolve continuously and the poorly-understood resilient cancer stem-cells repopulate continuously; they represent the main culprits of relapse. The vulnerability of developing drug-resistance using monotherapy (**Fig. 1A–C**) and the nature of these two culprits prompted us to exploit antitumor immunity to overcome relapse by investigating a new strategy of combining targeted therapy (IM) with immunotherapy (peginterferon α-2b [PegIntron®]) in GIST.[6] Our results show that this combination treatment is well tolerated, safe, and induced significant IFNγ-producing-CD8$^+$, -CD4$^+$, -NK cell, and robust IFNγ-producing-tumor-infiltrating-lymphocytes, signifying induction of innate and Th1 adaptive cell-mediated immunity (Th1 response).[6] Complete remission (CR) + partial response (PR) = 100%; overall survival = 100%; one patient died of unrelated illness while in radiographic near-CR; after a median follow-up of 3.9 y, five of the seven evaluable patients are in continuing PR/CR with duration > doubling the median-genotype-specific-PFS of the Phase III IM-monotherapy trial (CALGB150105/SWOGS0033)[2] (**Fig. 1D**, Pts#1, 2, 4, 5, 8); Pt#6 developed IM-resistance, but when peginterferon α-2b was re-initiated, a second PR was induced, indicative of recall of antitumor immunity.[6]

Interferon α (IFNα) is a Type 1 IFN, a physiological danger signal (3rd signal) and immune modulator.[7,8] Peginterferon α-2b and peginterferon α-2a (Pegasys®) are two currently available long-acting-IFNα. IFNα have been used to treat several hematological neoplasia, Kaposi sarcoma, and viral hepatitis in the past

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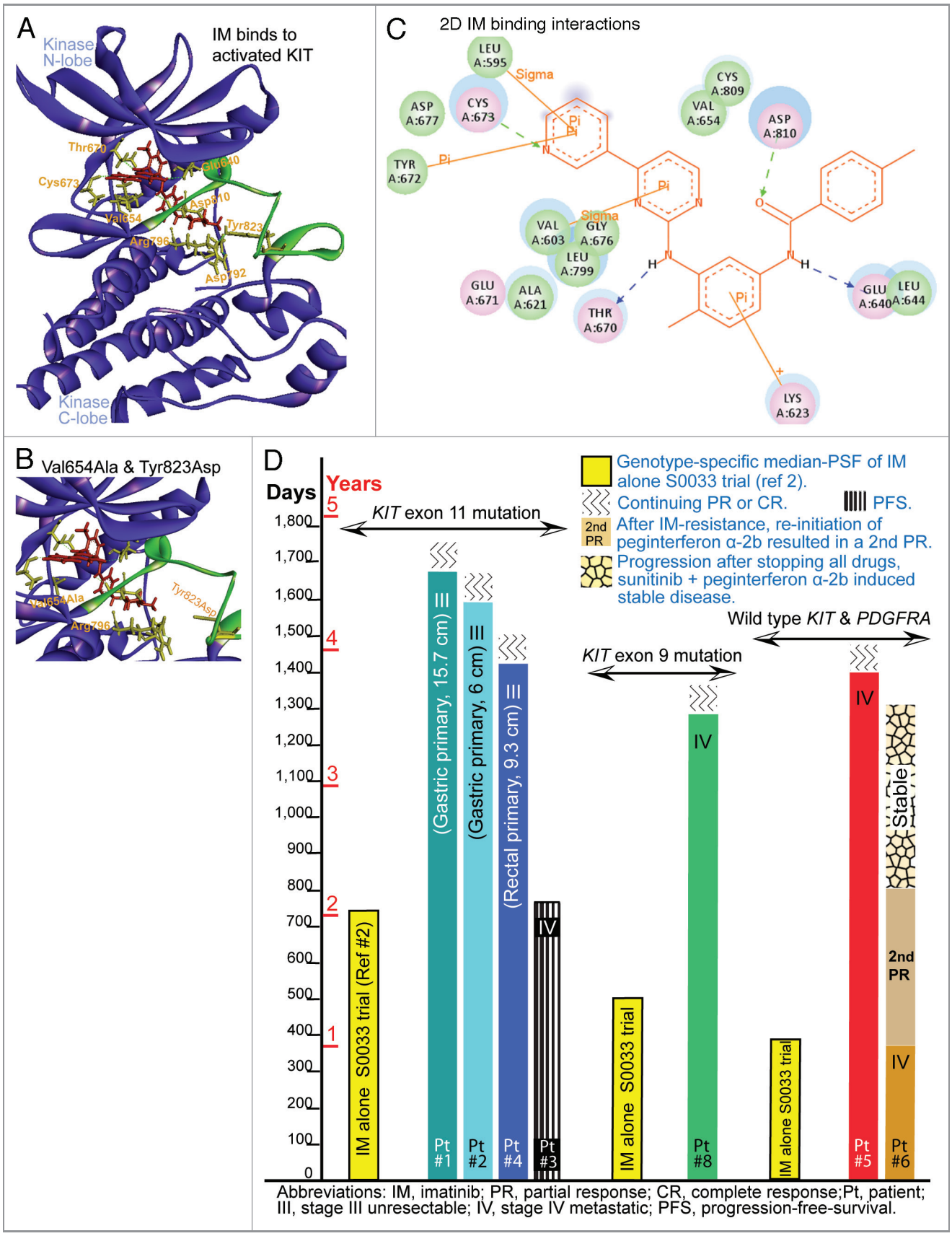

Figure 1. For figure legend, see page 775.

Figure 1 (See opposite page). IM binding to activated KIT (top panel) and remission duration post-combination treatment with IM plus peginterferon α-2b in GIST[6] (lower panel). (A-B), 3D ribbon diagram of IM binding to activated KIT. Red, IM; green, activation loop; green dotted line, hydrogen bonds; gold, key amino acids. IM forms critical hydrogen bonds to neighboring amino acids Thr670, Cys673, Glu640 and Asp810. Val654 is in a key position supporting the correct alignment of the key hydrogen bonds of IM to Thr670 and Asp810 (DFG motif). There are two additional critical hydrogen bonds, one between Arg796 and Tyr823 (activation loop), another one between Arg796 and Asp792 (A). Any amino acid substitution in these key positions can disrupt the critical conformation and impede IM binding. Two such examples, missense mutation Val654Ala and Tyr823Asp are shown (B) Val654Ala resulted in loss of hydrophobic support of IM, and missense mutation Tyr823Asp resulted in loss of hydrogen bonds with Arg796. (C) 2D binding interactions of IM with neighboring amino acids, hydrogen bonds are show as green (H-bond interaction with amino acid main chain) and blue (H-bond interaction with amino acid side chain) arrows. Pi stacking interactions are shown as orange lines. Residues involved in hydrogen-bond, charge or polar interactions are shown as pink circles. Residues involved in van der Waals interactions are shown as green circles. (D) Remission duration post-combination treatment after median follow-up of 3.9 y (4.6 – 3.5 y, calculated as of 02/15/2012), five patients are in continuing PR/CR (Pts#1, 2, 4, 5, 8), much improved comparing to the median-genotype-specific-PFS of Phase III S0033 IM monotherapy trial (yellow bars). Pt#3 presented with Stage IV aggressive GIST harboring *KIT* exon 11 deletion, extensive liver metastasis, mitotic figure ≥ 40/high power field, had swift excellent response achieving PR within 8 weeks, but progressed with PFS slightly longer than two years. Pt#6 developed IM-resistance, and re-initiation of peginterferon α-2b resulted in second PR. Pt#6 progressed again while off all treatment, and combination treatment of sunitinib plus peginterferon α-2b resulted in stable disease (B, last column).

50 years and have demonstrated good tolerability and safety. A minimum of ten steps are required to develop antitumor immunity: (1) immunogenic tumor kill;[9,10] (2) triggering innate antitumor immunity; (3) initiating adaptive antitumor response in the presence of 1st (tumor-specific antigens), 2nd (costimulation), and 3rd (danger) signals; (4) tumor-antigen capture and processing by dendritic cells (DCs) with differentiation toward Th1 response (not T regulatory response); (5) cross-priming by DCs in the context of MHC-I and costimulatory molecules to subsets of naive T-lymphocytes resulting in generation of tumor-specific T-lymphocytes, clonal expansion and differentiation in lymphoid organs; (6) effector T-lymphocytes trafficking to tumor sites; (7) cytokines production, especially IFNγ, by effector T-lymphocytes upon tumor antigen recognition, overcoming the suppressive tumor microenvironment; (8) effector phase consisting of effector functions of $CD4^+$ T-lymphocytes and $CD8^+$ T-lymphocytes (CTLs)—killing of tumor cells; (9) Differentiation into $CD4^+$- and $CD8^+$-memory T-lymphocytes; (10) apoptosis of tumor-antigen-activated T-lymphocytes to achieve homeostasis and minimize autoimmune disease. Steps 4 and 5 require > two weeks, and steps 8 may take > 6 mo because CTLs are usually overwhelmingly outnumbered by tumor cells (approximately $10^{8.5}$ tumor cells/cm^3). Our highly promising clinical outcome (**Fig. 1D**) testifies that IFNα successfully modulates steps 2–10 toward antitumor response during combination treatment.[6] Monoclonal antibodies (mAb) against cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4) (e.g., ipilimumab) can modulate step 4, and mAb against programmed cell death 1 pathway (PD-L1/PD-1) (e.g., MDX-1105) can modulate steps 8 and 10, and represent promising immune modulators to enhance CTL functions, but their adverse effects remains unknown contrasting the 5 years' experience with IFNα.

Effective, non-marrow suppressive, minimally toxic treatment modalities constitute "targeted therapy" in the broad sense, which includes targeted small molecule inhibitors, hormone receptor agonist/antagonists, and monoclonal antibodies. Advances in tumor biology undoubtedly will lead to discovery of more effective targeted therapeutic agents in the future, but drug-resistance and early relapse will undoubtedly maintain recurrent themes using monotherapy. We combined peginterferon α-2b (an immune modulator and a danger signal) with IM (non-marrow suppressive and effective in tumor killing to provide 1st and 2nd signals) in GIST model, demonstrated significant induction of innate and Th1 response along with highly promising clinical outcome[6] (**Fig. 1D**), and we plan to incorporate the insights gained into future larger trials. We believe this mew strategy may benefit large number of different subtypes of cancer patients. Currently, many malignancies have encouraging suitable "targeted" drugs to achieve simultaneous PR/CR and antitumor immunity if combined with immunotherapy (peginterferon α). The short PFS of vemurafenib-treated $B\text{-}RAF^{V600}$-mutated-melanoma[3] may be improved by combination with peginterferon α (immunotherapy) and warrant a trial. Another example is to combine oxaliplatin[10] (or irinotecan) with peginterferon α for colorectal cancer with one precaution—careful dosing of chemotherapeutic agents to avoid leukopenia, which is detrimental to DC differentiation toward Th1 response. Some other very encouraging examples to combine with peginterferon α (immunotherapy) and warrant testing include leuprolide and or bicalutamide for prostate cancer, tamoxifen or aromatase inhibitors for ER^+PR^+ breast cancer, lapatinib for ER^+HER2^+ breast cancer, trastuzumab for $HER2^+$ breast cancer, erlotinib or crizotinib for non-small cell lung cancers harboring EGFR mutations or *EML4-ALK* fusion gene respectively, and radiation therapy for radio-sensitive tumors (i.e., seminomas, Ewing sarcoma, lymphoma).

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