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Radotinib is an Effective Inhibitor of Native and Kinase Domain-Mutant BCR-ABL1

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Letter to the Editor

Philadelphia chromosome positive (Ph^+) leukemia is driven by the constitutive enzymatic activity of the BCR-ABL1 fusion kinase.[1] Tyrosine kinase inhibitors (TKIs) that block the activity of BCR-ABL1 are successfully used clinically to treat chronic myeloid leukemia (CML) and Philadelphia chromosome-positive acute lymphoblastic leukemia (Ph^+ ALL). The Food and Drug Administration (FDA) granted regulatory approval for the first of these, imatinib, in 2001. Emergence of clinical imatinib resistance, chiefly due to BCR-ABL1 kinase domain mutations, motivated the development and regulatory approval of new TKIs, including nilotinib, dasatinib, bosutinib and ponatinib.[1–3] One strategy, as exemplified by nilotinib (Fig. 1A), is rational design of imatinib derivatives with substantially higher binding affinity. Nilotinib is approved for first-line use in the U.S. and is an order of magnitude more potent than imatinib, which translates into improved inhibitory activity against many of the common BCR-ABL1 mutants.[4] The most important mutational liability is BCR-ABL1^{T315I}, which is completely insensitive to all approved TKIs except ponatinib.[1, 5]

Radotinib (IY5511HCl; Supect) is an oral, high-affinity BCR-ABL1 inhibitor that bears strong structural resemblance to imatinib and especially to nilotinib (Fig. 1A), and was approved in Korea for second-line CML treatment in 2012. One stated motivation for developing radotinib is to provide emerging geographic regions with a more affordable option compared to other second generation TKIs.[6, 7] An interim report on the efficacy and

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

MSZ, NAV and KCG performed experiments, analyzed data and prepared display items. NAV assisted in writing the manuscript. MSZ, MWD and TO conceptualized, planned and supervised the studies and wrote the manuscript.

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safety of radotinib in a phase II clinical trial enrolling chronic phase CML patients with resistance or intolerance to BCR-ABL1 TKIs, mostly imatinib, was recently released (clinicaltrials.gov identifier: 01602952).[7] At a minimum follow-up of 12 months and a median duration of follow-up of 2 years, the phase II clinical trial results suggest that radotinib is effective and well tolerated, with major and complete cytogenetic response rates comparable to nilotinib and dasatinib in similar patient populations.[8, 9] Our pre-clinical study was performed to gain a better understanding of the mutational liabilities associated with radotinib, currently in phase III clinical trials, and to better understand the binding mode of radotinib compared to the highly similar nilotinib.

A subset of patients (12/77; 16%) included in the report had one (10 patients) or two (2 patients) detectable BCR-ABL1 kinase domain mutations at baseline: M244V, M244V and H396R, G250E, Y253F and E355G, E255K, E255V, F317L, M351T, E355G, F359V (2 patients), and L387M (Table S1).[7] Our pre-clinical resistance-profiling panel includes 8 of the 10 mutated positions observed, with the exception of 355 and 387. The resistance profiles of radotinib and the five FDA-approved TKIs are compared in Fig. 2. In addition to radotinib being remarkably similar in structure to nilotinib, the two TKIs also have a similar resistance profile when examined via MTS assay using Ba/F3 cells expressing native BCR-ABL1, BCR-ABL1 single mutants, and BCR-ABL1 compound mutants. Our results forecast substantial resistance to radotinib for the above patients exhibiting a BCR-ABL1 mutation at any of the following positions: 250, 253, 255, 359. In contrast, mutations at positions 244, 317, 351 and 396 are predicted to confer little or no resistance to radotinib. Further follow-up on these patients was not available. Additionally, newly emergent mutations on therapy were detected in six patients: E255V (two patients), T315I, F317L, F359V, E459K. Four of these occurrences (E255V x 2, T315I, F359V) are predicted to result in high-level radotinib resistance. F317L is predicted to be sensitive to radotinib (Table S2). E459K was not evaluated in this study, but is reported to be moderately resistant to imatinib and sensitive to nilotinib.[4, 10, 11]

When comparing the nilotinib and radotinib IC_{50} values within a given cell line, radotinib was only more potent than nilotinib in 2 cell lines, but in both cases this was in the margin of error (native: 32.5 nM vs. 30.6 nM, and M244V: 55.6 nM vs. 50.8 nM). At the same time nilotinib was significantly more potent than radotinib in several single mutants, including: G250E (306.5 nM vs. 472.7 nM), Y253H (1719.3 nM vs. 2804.0 nM), E255V (897.2 nM vs. 1618.7 nM), V299L (74.4 nM vs. 106.4 nM), F317L (100.5 nM vs. 200.1 nM), and F359C (370.0 nM vs. 569.8 nM). Additionally, nilotinib was between 2 and 3 times more potent in 3 compound mutant expressing cell lines: G250E/V299L (347.3 nM vs. 737.5 nM), V299L/F317L (133.6 nM vs. 362.1 nM), and V299L/F359V (380.2 nM vs. 805.0 nM). Nilotinib was also >3 times more potent in 2 cell lines: E255V/V299L (4.1 fold; 1222.3 nM vs. 4982.0 nM), and F317L/F359V (14.4 fold; 622.9 nM vs. 8964.3 nM) (Figure 2A, B, and C; Table S3).

Immunoblot analysis confirmed that radotinib effectively blocks the tyrosine kinase activity of native BCR-ABL1 and the V299L, F317L, and M351T point mutants, while the E255V mutant exhibited residual enzyme activity at intermediate concentrations of radotinib, in line with cellular resistance profiling (Fig. 2D). The highest tested concentration of radotinib

(2000 nM) did not diminish the kinase activity of the T315I mutant. Immunoblot analysis confirmed that radotinib was effective against a subset of non-T315I compound mutants composed of individually sensitive mutations (e.g. V299L/F317L and V299L/M351T) but not compound mutants that included Y253H, E255V or F359V as a component (Figure 2E).

We investigated the binding modes of nilotinib and radotinib using Molecular Dynamics simulations (Fig. 1B). Modeling of radotinib into the ABL1 kinase domain revealed a similar but subtly different binding mode compared to that of nilotinib in the ABL1 crystal structure (PDB entry 3CS9). The two inhibitors bind nearly identically, with the important exception of the leftmost ring system in Figure 1A (boxed in red), pyridinyl in nilotinib as compared to pyrazinyl in radotinib. In the case of nilotinib, the pyridinyl nitrogen (N3) is positioned for optimal hydrogen bond interaction with residue M318 (Fig. 1C). However, the equivalent nitrogen (N5) in the pyrazinyl ring of radotinib is not used as the hydrogen bond acceptor for residue M318. Instead, docking simulation predicts that the other nitrogen (N2) is utilized in order to balance the beneficial hydrogen bonding interaction with minimization of steric clash between the ortho-nitrogen in the pyrazinyl ring and ABL1 residue Y253 (Fig. 1D).

Clinical trials for radotinib are ongoing[7], and a phase III trial evaluating radotinib as a first line agent is ongoing (clinicaltrials.gov identifier: 01511289). Despite its similarity to nilotinib, a driving force for the development of radotinib is that it may represent an affordable alternative to other TKIs. The dosing schedule for radotinib, as used in the phase II clinical trial, of 400 mg twice daily is identical to the recommended starting dose for nilotinib in the setting of resistance or intolerance.[8, 12] The recommended dose of nilotinib in the first line setting is 300 mg twice daily.[13] The pharmacokinetic parameters for radotinib remain to be established. If steady-state levels are substantially different than for nilotinib, this could influence the range of point mutants that are clinically controllable with radotinib. Another consideration is the safety profile. While nilotinib is generally well tolerated, a number of adverse events have been reported, such as headaches, skin rashes, hyperglycemia, elevated lipase and transaminases.[13] The types and severity of adverse events reported with radotinib appears similar. Longer follow-up will be required to determine whether radotinib shares the recently reported cardiovascular toxicity of nilotinib.[14] Overall, the *in vitro* efficacy of radotinib is comparable to that of nilotinib against native BCR-ABL1 and single BCR-ABL1 mutants, and this is reflected by the similar activity in patients with imatinib failure.[7] Radotinib has less *in vitro* activity than nilotinib against several compound mutants, which may translate into reduced clinical activity in patients with these mutations. This study provides a detailed summary, from a mutated BCR-ABL1 perspective, of how radotinib fits into the already available TKI arsenal. An in-depth understanding of the binding mode of radotinib and nilotinib, as well as a complete *in vitro* TKI resistance profile is particularly important due to high structural similarity between radotinib and nilotinib.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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References

1. O'Hare T, Zabriskie MS, Eiring AM, Deininger MW. Pushing the limits of targeted therapy in chronic myeloid leukaemia. Nat Rev Cancer. 2012; 12:513–526. [PubMed: 22825216]
2. Gorre ME, Ellwood-Yen K, Chiosis G, Rosen N, Sawyers CL. BCR-ABL point mutants isolated from patients with imatinib mesylate-resistant chronic myeloid leukemia remain sensitive to inhibitors of the BCR-ABL chaperone heat shock protein 90. Blood. 2002; 100:3041–3044. [PubMed: 12351420]
3. Shah NP, Nicoll JM, Nagar B, Gorre ME, Paquette RL, Kuriyan J, et al. Multiple BCR-ABL kinase domain mutations confer polyclonal resistance to the tyrosine kinase inhibitor imatinib (STI571) in chronic phase and blast crisis chronic myeloid leukemia. Cancer Cell. 2002; 2:117–125. [PubMed: 12204532]
4. Weisberg E, Manley PW, Breitenstein W, Bruggen J, Cowan-Jacob SW, Ray A, et al. Characterization of AMN107, a selective inhibitor of native and mutant Bcr-Abl. Cancer Cell. 2005; 7:129–141. [PubMed: 15710326]
5. O'Hare T, Shakespeare WC, Zhu X, Eide CA, Rivera VM, Wang F, et al. AP24534, a pan-BCR-ABL inhibitor for chronic myeloid leukemia, potently inhibits the T315I mutant and overcomes mutation-based resistance. Cancer Cell. 2009; 16:401–412. [PubMed: 19878872]
6. Experts in Chronic Myeloid L. The price of drugs for chronic myeloid leukemia (CML) is a reflection of the unsustainable prices of cancer drugs: from the perspective of a large group of CML experts. Blood. 2013; 121:4439–4442. [PubMed: 23620577]
7. Kim SH, Menon H, Jootar S, Saikia T, Kwak JY, Sohn SK, et al. Efficacy and safety of radotinib in chronic phase chronic myeloid leukemia patients with resistance or intolerance to BCR-ABL1 tyrosine kinase inhibitors. Haematologica. 2014; 99:1191–1196. [PubMed: 24705186]
8. Kantarjian H, Giles F, Wunderle L, Bhalla K, O'Brien S, Wassmann B, et al. Nilotinib in imatinib-resistant CML and Philadelphia chromosome-positive ALL. N Engl J Med. 2006; 354:2542–2551. [PubMed: 16775235]
9. Talpaz M, Shah NP, Kantarjian H, Donato N, Nicoll J, Paquette R, et al. Dasatinib in imatinib-resistant Philadelphia chromosome-positive leukemias. N Engl J Med. 2006; 354:2531–2541. [PubMed: 16775234]
10. Hughes T, Saglio G, Branford S, Soverini S, Kim DW, Muller MC, et al. Impact of baseline BCR-ABL mutations on response to nilotinib in patients with chronic myeloid leukemia in chronic phase. J Clin Oncol. 2009; 27:4204–4210. [PubMed: 19652056]
11. Apperley JF. Part I: mechanisms of resistance to imatinib in chronic myeloid leukaemia. Lancet Oncol. 2007; 8:1018–1029. [PubMed: 17976612]
12. Kantarjian HM, Giles FJ, Bhalla KN, Pinilla-Ibarz J, Larson RA, Gattermann N, et al. Nilotinib is effective in patients with chronic myeloid leukemia in chronic phase after imatinib resistance or intolerance: 24-month follow-up results. Blood. 2011; 117:1141–1145. [PubMed: 21098399]
13. Saglio G, Kim DW, Issaragrisil S, le Coutre P, Etienne G, Lobo C, et al. Nilotinib versus imatinib for newly diagnosed chronic myeloid leukemia. N Engl J Med. 2010; 362:2251–2259. [PubMed: 20525993]
14. Kim TD, le Coutre P, Schwarz M, Grille P, Levitin M, Fateh-Moghadam S, et al. Clinical cardiac safety profile of nilotinib. Haematologica. 2012; 97:883–889. [PubMed: 22271904]
15. Sastry GM, Adzhigirey M, Day T, Annabhimoju R, Sherman W. Protein and ligand preparation: parameters, protocols, and influence on virtual screening enrichments. J Comput Aid Mol Des. 2013; 27:221–234.

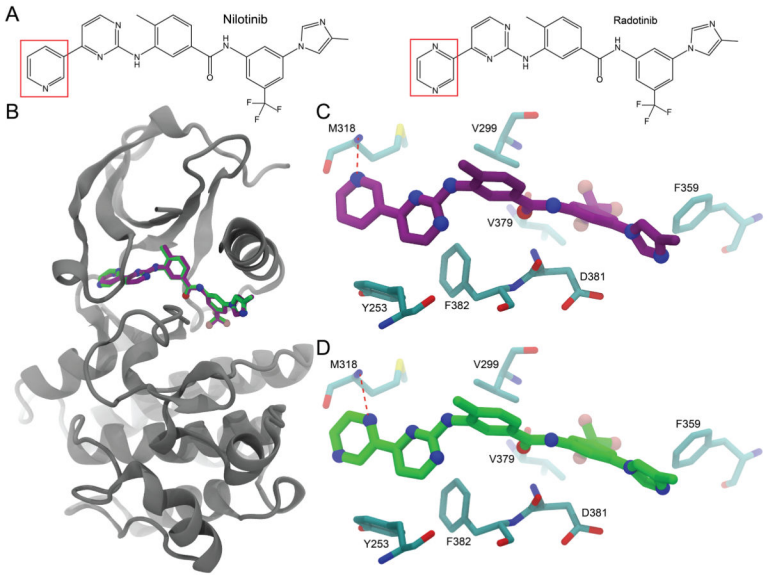

Figure 1. Docking simulations of radotinib identify a different binding mode than nilotinib

(**A**) The chemical structures of nilotinib and radotinib. The box indicates the region in which these TKIs are structurally distinct. The chemical designation for nilotinib is 4-methyl-N-[3-(4-methyl-1H-imidazol-1-yl)-5-(trifluoromethyl)phenyl]-3[[4-(3-pyridinyl)-2-pyrimidinyl]amino]-benzamide. The chemical designation for radotinib is 4-methyl-N-[3-(4-methyl-1H-imidazol-1-yl)-5-(trifluoromethyl)phenyl)-3-((4-pyrazin-2-yl)pyrimidin-2-yl)amino)benzamide. (**B, C, and D**) The inactive conformation of the BCR-ABL kinase in complex with nilotinib was chosen for docking simulation purposes. The crystal structure (PDB entry 3CS9)[4] was prepared by removing nilotinib from the crystal structure and hydrogen atoms were added using Schrödinger Protein Preparation tool (Schrödinger LLC, New York, NY, 2012).[15] Prime module was invoked to build any side-chain atoms missing in the crystal structure. All possible protonation and tautomer states were generated (apparent pH in the range 7.0 ± 2.0). The position of the hydrogen atoms was further refined by minimizing the structure with heavy atoms restrained using the OPLS-AA force field to a maximum atom-positional root-mean-square deviation (RMSD) of 0.3 Å. Docking calculations were performed using Glide (Schrödinger, LLC) and a scoring grid was precomputed by placing an outer cubical box of length 22 Å and an inner box of length 14 Å centered at the nilotinib binding site. The hydroxyl groups of all Ser, Thr, and Tyr residues in the vicinity of the binding site were allowed to be flexible during the grid generation process. Chemical structures of radotinib and nilotinib were sketched using Maestro program and minimized using LigPrep module (version 2.5) of the Schrödinger program to generate the low-energy conformation. Docking calculations were performed in extra precision (XP) mode (version 5.7). (**B**) Binding mode of radotinib to ABL1 kinase domain. Calculated energy-minimized binding pose of radotinib (green) overlaid on solved crystal structure of nilotinib (purple) bound to the ABL1 kinase domain. (**C**) Hydrogen bonding network and key electrostatic interactions between nilotinib and ABL1 kinase domain. (**D**) Hydrogen bonding network and key electrostatic interactions between radotinib and ABL1 kinase domain.

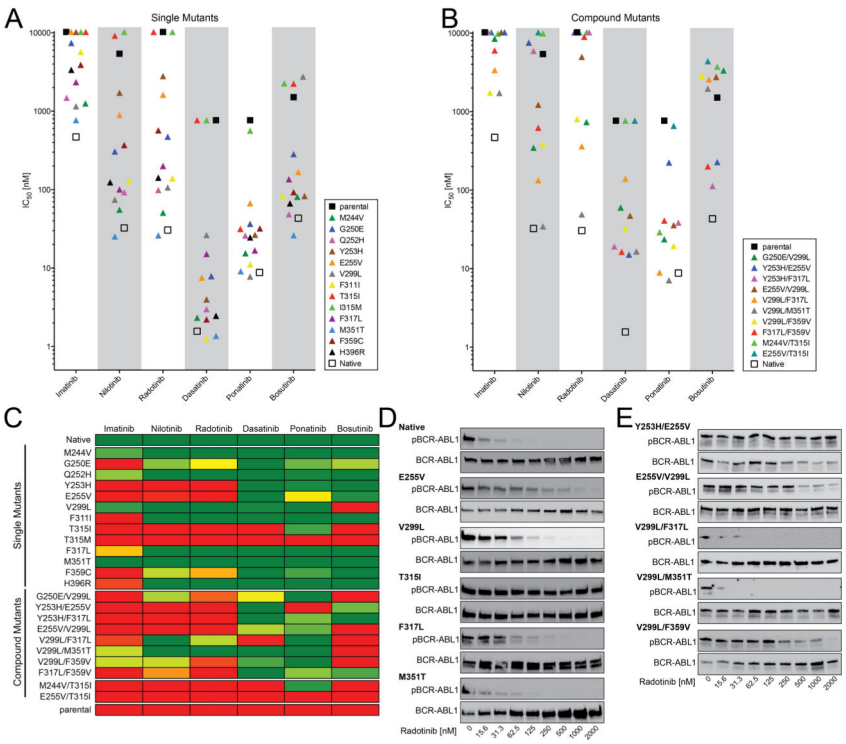

Figure 2. BCR-ABL1 mutant sensitivity profile to radotinib and 5 approved TKIs

(**A–B**) Ba/F3 BCR-ABL1-expressing cells were distributed into 96-well plates (2×10^3 cells/well) and incubated in 2-fold escalating concentrations of dasatinib, ponatinib (0–768 nM), imatinib, nilotinib, radotinib, or bosutinib (0–10,240 nM) for 72 hours. Proliferation was assessed by methanethiosulfonate (MTS)-based viability assay (CellTiter 96 AQueous One; Promega). IC_{50} values are reported as the mean of three independent experiments performed in quadruplicate. Cell proliferation IC_{50} values of TKIs against BCR-ABL1 (**A**) single mutants, (**B**) compound mutants. Mean IC_{50} values are plotted (see Table S3). (**C**) Heat map of TKI IC_{50} values for single and compound mutants. A color gradient from green (sensitive) to yellow (moderately resistant) to red (highly resistant) denotes the IC_{50} sensitivity to each TKI: imatinib (green: <1000 nM; yellow: 1000–4000 nM; red: >4000 nM); nilotinib (green: <200 nM; yellow: 200–1000 nM; red: >1000 nM); radotinib (green: <200 nM; yellow: 200–1000 nM; red: >1000 nM); dasatinib (green: <25 nM; yellow: 25–150 nM; red: >150 nM); ponatinib (green: <25 nM; yellow: 25–150 nM; red: >150 nM); bosutinib (green: <150 nM; yellow:150–1000 nM; red: >1000 nM). (**D, E**) Ba/F3 cells expressing (**D**) native, single mutant or (**E**) compound mutant BCR-ABL1 were cultured for 4 hours in standard medium alone or with escalating concentrations of radotinib. Following radotinib exposure, cells were lysed (0°C; 30 min.) in 30 μL RIPA buffer (150 mM NaCl, 1% NP40, 0.1% SDS, 1 M Tris [pH 8.0]) containing protease (Complete Mini, Roche) and phosphatase (PhosStop, Roche) inhibitors. Samples were denatured by boiling for 10 min in SDS-PAGE loading buffer. Lysates were separated on 4–15% Tris-glycine gels, transferred, and immunoblotted with antibodies for the BCR N-terminus (3902; Cell Signaling Technology) and phospho-ABL1 (Y393 [1a numbering]; Cell Signaling Technology).