DYNAMICS OF EXPANSION OF TYROSINE KINASE INHIBITOR-RESISTANT MUTANTS AS ASSESSED BY DEEP SEQUENCING OF THE BCR-ABL KINASE DOMAIN: IMPLICATIONS FOR ROUTINE MUTATION TESTING

Konference: 2013 18th Congress of the European Hematology Association - účast ČR

Kategorie: Maligní lymfomy a leukémie

Téma: Drug responsiveness in acute leukemias

Číslo abstraktu: S608

Autoři: Simona Soverini; Caterina De Benedittis; Mgr. Kateřina Machová (Poláková), Ph.D.; Ing. Adéla Benešová (Broučková), Ph.D.; M.D. Cristina Papayannidis; Maria Chiara Abbenante; Ilaria Iacobucci, PhD; Claudia Venturi; Anna Ferrari; Federica Cattina; Prof. Domenico Russo; Mario Luppi; Paola Bresciani; MD Antonella Vitale; Robert (Robin) Foa (Foà); MD Michele Baccarani; MD Michele Cavo; Prof.MD Giovanni Martinelli

Background:

In Philadelphia-positive (Ph+) acute lymphoblastic leukemia (ALL) patients (pts), efficacy of tyrosine kinase inhibitor (TKI)-based therapies is often compromised by selection of resistant mutations in the BCR-ABL kinase domain (KD). Currently, the gold standard for BCR-ABL KD mutation screening is conventional Sanger sequencing (SS). However, more sensitive approaches are desirable to allow more timely and rational therapeutic intervention.

Aims:

A Deep sequencing (DS) strategy based on the Roche 454 next-generation sequencing technology was set up in order to: study the dynamics of expansion of different types of BCR-ABL KD mutations in Ph+ ALL patients developing resistance to TKI-based therapies; test the ability of DS to highlight emerging clones harboring TKI-resistant mutations.

Methods:

29 Ph+ ALL pts who had developed resistance to TKI-based (imatinib, dasatinib, nilotinib) therapies were selected for this retrospective analysis. All the pts were known to have developed TKI-resistant BCR-ABL mutations on treatment, as assessed by SS. To reconstruct the dynamics of mutation emergence, longitudinal re-analysis of samples from relapse backwards (n=97; 1-3 months sampling interval) was performed on a Roche GS Junior instrument. DS runs were designed so as to enable high sensitivity mutation calling (minimum target sequence coverage 4,000 reads). However, to minimize the likelihood of false positive results, data were analyzed filtering out all variants with <1% abundance.

Results:

DS could successfully detect all the mutations (n=85) previously identified by SS (>15% abundance). In addition, DS revealed that both those samples that had been scored as apparently wild-type by SS and those samples already known to harbor mutations as assessed by SS might be carrying one or more 'lower level' mutations (<15% abundance). In the latter cases, clonal analysis showed complex textures with the same mutation alone and also in combination with other(s) ('compound' mutations) in distinct subclones. Some lower level mutations were silent or apparently irrelevant from a clinical standpoint (passenger mutations?). In more than half of the cases, however, known TKI-resistant variants could be recognized that corresponded either to 'withdrawing' mutants not (yet) entirely de-selected by the switch in TKI or to outgrowing mutations anticipating an imminent relapse. Lower level mutations were confirmed with independent methods (ASO-PCR, RFLP). Notably, in 16/29 (55%) pts with molecularly detectable disease but not yet evidence of cytogenetic or hematologic relapse, DS could identify emerging mutations 1 to 3 months before they became detectable by SS. In the remaining 13 pts, however, outgrowth of the TKI-resistant mutation (T315I=7, Y253H=2, E255K=2, E255V=1 and F317L=1) was so rapid that not even a strict monthly monitoring could have allowed to pick them up before they became dominant.

Summary / Conclusion:

Now that multiple options are available, BCR-ABL KD mutation monitoring is a precious tool to maximize the efficacy of TKI-based regimens as induction or salvage therapy of Ph+ ALL. DS proved as reliable as SS for the detection of mutations with >15% abundance. As a key advantage, DS added precious quantitative and qualitative information on the full repertoire of mutated populations, that SS underestimated in more than half of the samples analyzed. TKI-resistant mutations leading to patient relapse were not necessarily preexisting at diagnosis or at the time of switchover to another TKI, underlining the importance of regular monitoring of pts. Although the majority of mutations were found to arise and take over very rapidly, a monthly monitoring by our DS approach would have allowed to identify them earlier than SS actually did - and well in advance of clinical relapse - in half of the pts. DS technologies would enable higher sensitivity mutation calling: further studies are warranted to determine the optimal lower detection

limit to aim to in order to exclude both transient mutant subclones that will never take over and sequencing errors.

Supported by Fondazione CARISBO, PRIN, IGA MZCR NT11555

Abstrakta v časopise Haematologica 2013, Suppl1

Online Program

Datum přednesení příspěvku: 15. 6. 2013