COMPLEXITIES IN GENETIC TESTING FOR ALLOGENEIC BONE MARROW TRANSPLANT RECIPIENTS AND PATIENTS WITH HEMATOLOGIC MALIGNANCIES

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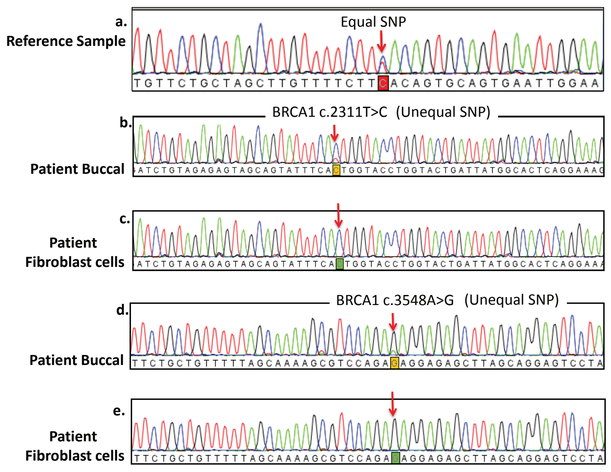
BACKGROUND

- Allogeneic bone marrow transplant (allo-BMT) recipients and patients with a hematologic malignancy (HM) present unique challenges when their blood/buccal samples are submitted for genetic testing.
- White blood cells from allo-BMT recipients are usually entirely derived from the transplant donor. Buccal samples from such patients can be chimeric, containing cells derived from both the recipient and the bone marrow donor.
- Therefore, genetic testing performed on DNA derived from blood/buccal samples of allo-BMT patients may reflect the genetic status of the transplant donor and not the recipient.
- Blood/buccal samples from individuals with a HM may demonstrate the presence of somatic mutations caused by the HM, which are not heritable.
- Genetic testing of blood/buccal samples in these individuals may therefore reflect the genetic status of the leukemic cells rather than the germline genetic status of the patient.

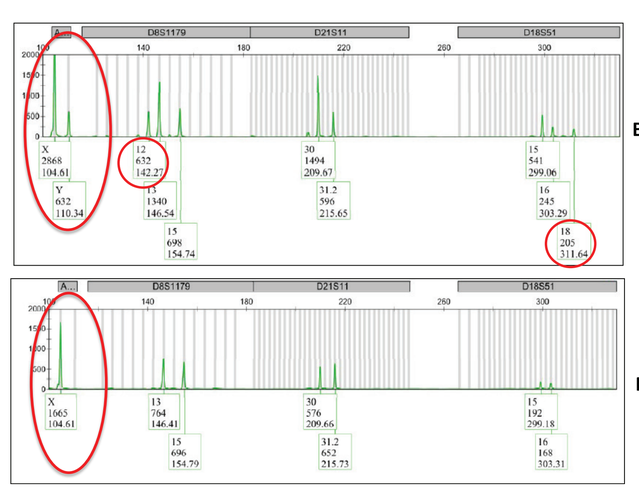
CLINICAL CASE 1: ATYPICAL SANGER SEQUENCING RESULTS ON A BUCCAL SAMPLE

- Female patient submitted a buccal sample for *BRCA1/BRCA2* testing.
- Sequencing analysis detected two unequal SNPs in *BRCA1.*
- Genotyping (STR) analysis revealed a genotypically male profile.
- Determined that patient received an allogeneic bone marrow transplant from a male donor.
- Re-tested patient using cultured fibroblast cells, which demonstrated an absence of the originally detected SNPs and a genotypically female profile on STR analysis.
- Therefore, buccal samples can show a detectable amount of donor contamination.



Panels b and d: Two missense changes are detected in the buccal sample, c.2311T>C and c.3548A>G, but the peaks representing the WT bases (red and green for thymine and alanine, respectively) are barely detectable.

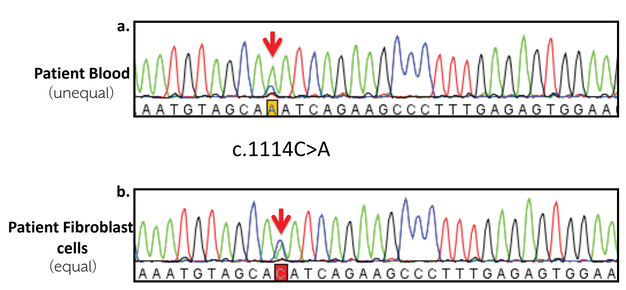
Panels c and e: The contaminating peaks are no longer present in the fibroblast sample. The patient is homozygous for both polymorphic SNPs.



Panel a. STR genotyping analysis of the patient's buccal sample detected a minor allele at several sites (red circles). This female patient appears to be genotypically male.
Panel b. There is no longer evidence of a minor allele at any locus in the fibroblast sample. The patient is now correctly demonstrated as genotypically female (red circle).

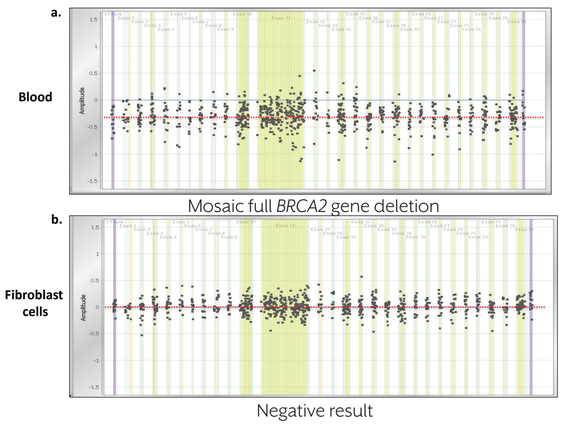
CLINICAL CASE 2: ATYPICAL RESULTS ON SANGER SEQUENCING AND TARGETED MICROARRAY CGH

- Patient submitted a blood sample for *BRCA1/BRCA2* testing.
- Sequencing analysis detected several unequal SNPs in *BRCA2.*
- Large rearrangement analysis by targeted microarray CGH detected a mosaic full gene deletion of *BRCA2.*
- Determined that patient was diagnosed with chronic lymphocytic leukemia (CLL).
- Re-tested patient using fibroblast cells, which demonstrated an absence of the full gene deletion and the presence of now balanced SNPs.
- FISH analysis demonstrated a loss of 13q14, the chromosomal region for *BRCA2.*



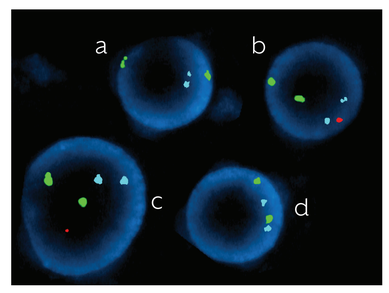
Panel a: A missense change was detected in *BRCA2*, c.1114C>A, in the patient's blood sample but the peak representing the WT base (cytosine, in blue) is barely detectable.

Panel b: The *BRCA2* WT and mutant peaks in the patient's fibroblast sample are now equal in height.



Panel a: For a heterozygous full gene deletion, the microarray probe cluster for the affected exons should center at approximately -0.5 on the Y-axis. All the probe clusters for the affected exons center at approximately -0.3 in the patient's blood sample (red dotted line). This patient is mosaic for a *BRCA2* full gene deletion.

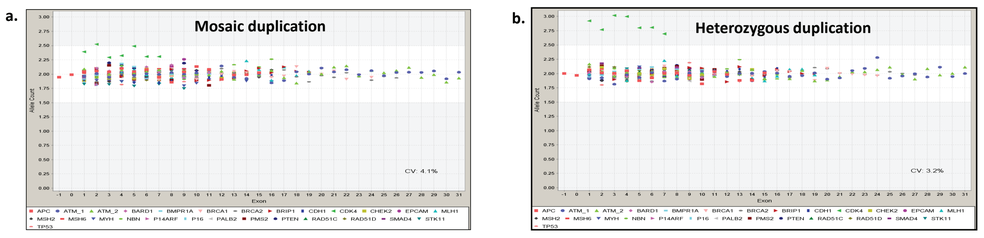
Panel b: The apparent deletion of all of *BRCA2* is not present in the patient's fibroblast sample. All of the probe clusters center at 0 (red dotted line), which is normal.



FISH analysis of the patient's blood sample detected only one copy of the 13q14 region (red) in two cells (b and c) and no copies in two others (a and d). The patient showed normal copy numbers for chromosome 12 (green) and the control region (LAMP locus) at 13q34 (aqua).

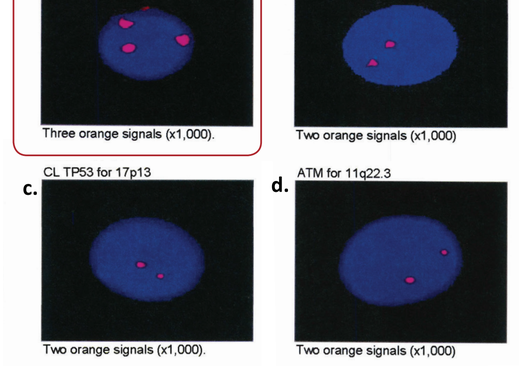
CLINICAL CASE 3: ATYPICAL DUPLICATION ON NEXT GENERATION SEQUENCING (NGS)

- Patient submitted a blood sample for 25-gene panel testing.
  - The NGS assay is able to detect sequencing mutations and large rearrangements (e.g. large duplications or deletions)
- Large rearrangement analysis by NGS detected a mosaic full gene duplication of *CDK4.*
- Determined that patient was diagnosed with CLL.
- FISH analysis demonstrated trisomy 12 in blood, the chromosome on which *CDK4* resides.



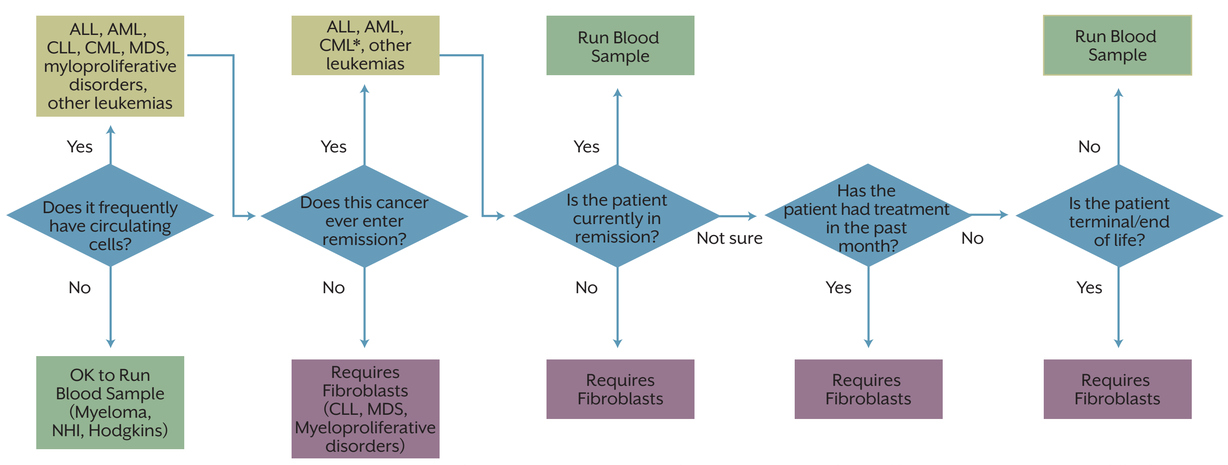
Panel a: In NGS large rearrangement results, exons at WT dosage should sit at ~2.00 (2 copies), deleted exons at ~1.00 (1 copy), and heterozygously duplicated exons should sit at ~ 3.00 (3 copies). All *CDK4* exons (green triangles) for this patient are located between 2.25-2.5. This patient carries an additional copy of *CDK4* in a proportion of her blood cells.

Panel b: A different patient with an additional copy of CDK4 in all cells is shown for comparison.



FISH analysis of the patient's blood sample shows three copies of chromosome 12 (Panel a). Only normal copy numbers are observed for loci representing chromosomal regions 13q14.3, 17p13 and 11q22.3 (Panels b, c and d).

SAMPLE TYPE DECISION TREE FOR HEMATOLOGIC CANCERS



*CML patients require documentation of molecular remission in the form of a PCR result on blood showing less than 0.01% of cancer cells.

CONCLUSIONS

- Genetic testing using blood/buccal samples from allo-BMT and HM patients may impact the interpretation of test results and subsequent clinical management of the patient.
- Cultured cells derived from skin fibroblasts are the most suitable sample type for allo-BMT patients, which is in accordance with the published 2012 NCCN Genetic/Familial High-Risk Assessment: Breast and Ovarian guidelines.
- We recommend submission of fibroblasts for HM patients as well, in cancer subtypes where the leukemic cells are circulating at a quantity that is detectable by our genetic analyses.
- It is imperative that the appropriate sample type be submitted for genetic testing so that the results reflect the heritable mutation status of the patient being tested.
- Mutations detected in the blood/buccal samples of these patients would be inappropriate for family testing, as it may result in false assurance for a family member who is negative for a mutation that he/she would not have been predisposed to inherit.

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