P2.19: Pilot study characterizing the combined clinical actionability of multiplexed germline and tumor biomarkers.

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BACKGROUND

A core tenet of precision medicine is that predictive biomarkers can enhance therapeutic decision-making. Such biomarkers are of germline and somatic lineage and can be considered to exist at three different levels of established clinical validity: 1) clinically endorsed (i.e., FDA approved), 2) clinically observed (i.e., predictive effect observed in patients), or 3) translational (i.e., supported by pre-clinical or computational evidence). Little is known about the prevalence and interdependence of such biomarkers in cancer patients.

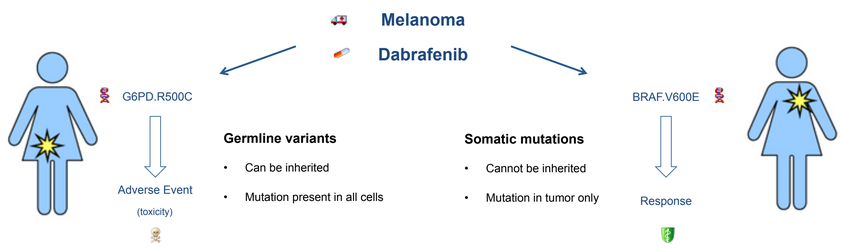


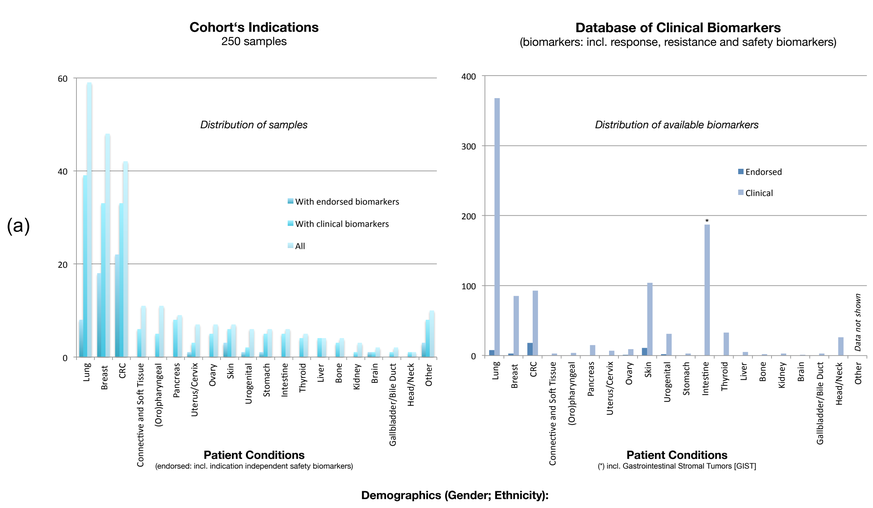
Figure 1: ***Biomarkers of somatic and of germline lineage.*** It is important to not characterize only somatic aberrations, as it is possible that patients' germline mutations might interfere with underlying molecular mechanisms and drug pharmacokinetics. Biomarker based analysis should consider both in combination and examine their interdependence.

Clinical validity	Evidence levels
Clinically endorsed	FDA approved
Clinically observed	Predictive effect observed in patients
Translational	Predictive effect supported by pre-clinical or computational evidence

Table 1: ***Established clinical validity levels for biomarkers.*** Treatment-relevant biomarkers can indicate resistance, response, and also safety issues. Current analysis covers not only such FDA approved (endorsed) biomarkers but also spans over a range of clinically validated evidence, acquired across a variety of cancer indications.

METHODS

We analyzed a randomly selected set of 250 patients with solid tumors, encompassing 20 different cancer indications. Biomarkers based on SNVs (tumor variant frequency ≥ 10% and average base calling quality score ≥ 25), INDELs and fusion proteins were identified using a 613 gene NGS panel and an analytical platform that screens identified aberrations against > 5,500 peer-reviewed predictive biomarkers. Identified biomarkers were then classified according to lineage, clinical validity/ evidence level, prevalence and potential functional interdependence.



(a)

(b)

Demographics (Gender; Ethnicity):

Sex vs Ethnicty	African	Latin American	Asian	European / American of European ancestry	Other or Unknown
Male	4	3	4	87	7
Female	16	7	6	111	5

Figure 2: ***Overview of cohort.*** The analysis of the set features potential combinations of genomic conditions and specific cancer-types that when characterized could reflect sufficient biomarker intricacies regarding disease mechanisms and potential therapeutic options.
(a) Dissemination of the cohort with respect to patient conditions; the samples cover a range of many common cancers; most represented in the set are *Lung*, *Breast* and *Colorectal* neoplasms,
(b) Dissemination of the cohort with respect to basic demographic attributes; the cohort contains samples that span across different types of ethnicity origins.

RESULTS

Predictive biomarkers were detected in 87.6% of tumors. Overall, 23.2% of the samples contained FDA endorsed biomarkers, while 69.2% contained clinically emergent biomarkers and 72% contained translational level biomarkers. Interestingly, we identified many cases where endorsed germline biomarkers predicting drug toxicity, modify the treatment conclusions drawn from somatic response biomarkers alone, due to toxicity concerns with a targeted therapy or its likely chemotherapy combination partner.

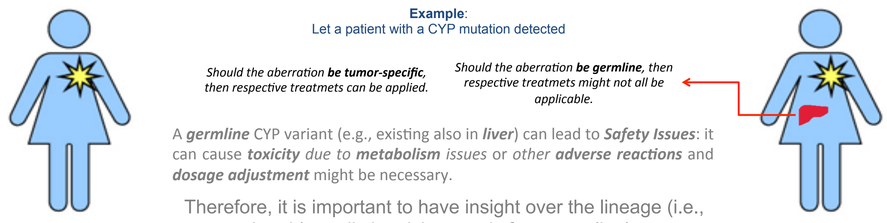


Figure 3: ***The issue of lineage.*** An example of why characterizing the combined clinical actionability of multiplexed germline and tumor biomarkers is important. These observations (see also Fig. 4 and 5) underscore the importance of comparing tumor with normal samples, as well as using carefully prepared (curated) biomarker databases that capture such lineage information.

The majority of identified aberrations were SNVs (detected in all patients); only in 6.8% and in 3.6% of samples were there fusions and INDELs identified, respectively:

- While sequencing normal tissue (blood), generally expects to give rise to SNVs that fall into one of three bins (0%, 50%, or 100%, depending on whether they're heterozygous or homozygous), dealing with tumor-only samples can be more complicated due to a variety of additional factors.
- These can for example include mixing normal with tumor cells, the existence of subclonal variants, Copy Number Variants, loss of heterozygocity or ploidy changes.

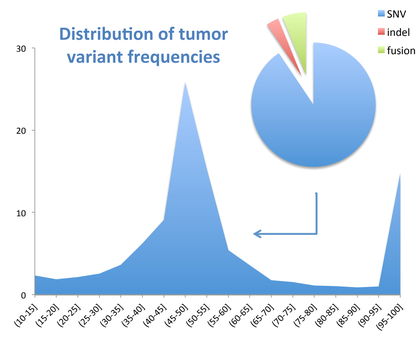
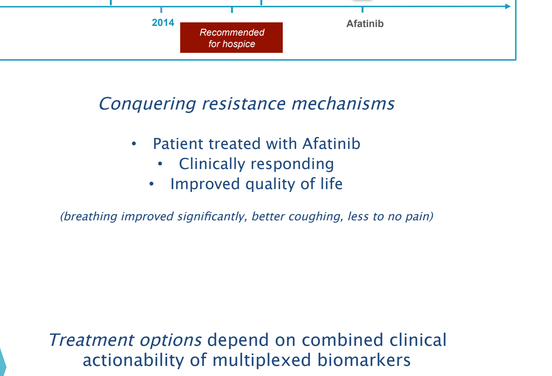


Figure 4: ***Overview of aberrations in the samples of the cohort.*** While the majority of detected aberrations were SNVs, handling tumor only samples can be ambiguous regarding the identification of which variants are somatic and which germline.



Samples with endorsed or with somatic clinical and pre-clinical biomarkers (219)

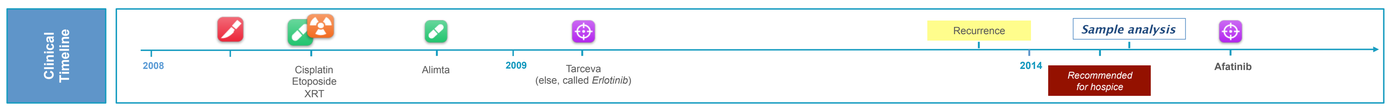
Figure 5: ***Dissemination of samples with respect to the clinical validity of identified biomarkers.*** The illustration depicts how clinical & pre-clinical biomarkers of somatic lineage appear across the samples of the cohort. In several cases, multiplexed combinations of evidence from different clinical validity levels have to be considered for the same patient (e.g., see overlap with samples that contain endorsed biomarkers).

Case Studies

It is essential to look at different levels of clinical validity, lineage and evidence levels to provide optimal treatment recommendations

Patient 1:

- Metastatic non-small cell lung cancer (NSCLC) patient



Predictive biomarker	Description
EGFR.ELR746	**FDA endorsed** biomarker (**response** for Erlotinib in NSCLC)
EGFR.pT790M	**Clinically** observed (evidence for Erlotinib) to confer **resistance**
EGFR exon 19 deletions	**FDA approved** Afatinib for the 1st line treatment of NSCLC patients with this profile

Conquering resistance mechanisms

- Patient treated with Afatinib
- Clinically responding
- Improved quality of life

(breathing improved significantly, better coughing, less to no pain)

Patient 2:

- Non-small cell lung cancer (NSCLC) patient

Predictive biomarker	Lineage	Effect explanation	Erlotinib	Gefitinib	Afatinib
EGFR.pL858R	somatic	Endorsed in patient indication	response	---	response
EGFR.pA871G	somatic	Clinical evidence (mechanism not clear)	resistance	---	---
ABCG2.pQ141K	germline	Indicates toxicity in patient indication	---	Adverse Event/Dosage Adjustment	---

Treatment options depend on combined clinical actionability of multiplexed biomarkers

germline vs tumor | safety, resistance, response | clinical vs endorsed

Patient 3:

- Urinary bladder (urogenital) neoplasms patient

Predictive biomarker	Description
TPMT.pY240C	**FDA endorsed** *germline* biomarker (P51580) predictive of ***adverse events*** for indication

While ***cisplatin*** could be ***indicated*** for the patient, there is a **germline safety** biomarker observed

CONCLUSIONS

- We have characterized the prevalence and clinical validity of predictive biomarkers in 250 patient samples.
- Predictive biomarkers are detected at a broad range of clinical validities.
- Our results confirm that somatic mutation profiling is essential but not sufficient for predicting cancer drug response, thereby supporting the need for diagnostic analysis of both germline and tumor biomarker information.