Blood-based multiplex kinase activity profiling as a predictive marker for clinical response to checkpoint blockade in advanced melanoma

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Presented at the American Society of Clinical Oncology Annual Meeting • Chicago, Illinois • June 1 - 5, 2018 ASCO

Abstract ID: 9578

Background

- There is an urgent need for response prediction to checkpoint inhibitor therapies.
- A significant proportion of patients does not benefit from the treatment, agents are costly and may cause serious toxicity.
- Kinase activity of peripheral blood cells (PBMCs) may reflect biological mechanisms underlying response to immunotherapy.

	Cohort 1 (n=10)	Cohort 2 (n=29)	Cohort 3 (n=33)	Cohort 4 (n=33)	Cohort 5 (n=38)	Total (n=143)
Gender						
Male	6 (60%)	13 (45%)	18 (55%)	22 (67%)	22 (58%)	81 (57%)
Female	4 (40%)	16 (55%)	15 (45%)	11 (33%)	16 (42%)	62 (43%)
Age at start (years)						
Mean (±SD)	59,4 (±15.2)	58,6 (±12.9)	62,7 (±10.7)	62,7 (±14.3)	60,7 (±14.2)	61,1 (±13.2)
Therapy						
Nivolumab	-	-	1 (3%)	2 (6%)	14 (37%)	17 (12%)
Pembrolizumab	-	-	30 (91%)	31 (94%)	23 (61%)	84 (59%)
Ipilimumab	10 (100%)	29 (100%)	-	-	-	39 (27%)
Nivo + ipi	-	-	2 (6%)	-	1 (3%)	3 (2%)
Number of pre-treatment lines						
None	3 (30%)	12 (41%)	20 (61%)	-	30 (79%)	65 (45%)
1	6 (60%)	8 (28%)	11 (33%)	17 (52%)	7 (18%)	49 (34%)
2	1 (10%)	-	2 (6%)	9 (27%)	1 (3%)	13 (9%)
3	-	-	-	7 (21%)	-	7 (5%)
Unknown	-	9 (31%)	-	-	-	9 (6%)
Pre-treatment with immunotherapy						
Yes	-	2 (7%)	2 (6%)	33 (100%)	2 (5%)	39 (27%)
No	10 (100%)	18 (62%)	29 (88%)	-	36 (95%)	93 (65%)
Unknown	-	9 (31%)	2 (6%)	-	-	11 (8%)

Table 1. Descriptive characteristics of advanced melanoma patients

Methods

- In a multi-center effort, data were prospectively collected from anti-CTLA4- or anti-PD1 treated advanced melanoma patients (n=143; table 1).

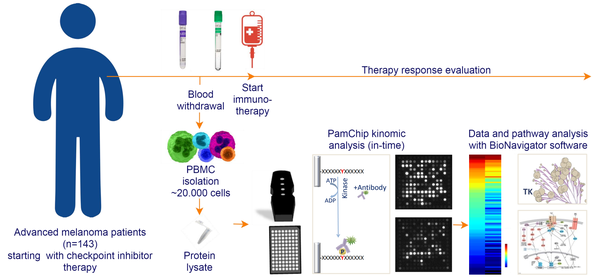

Figure 1. Kinase activity is measured in baseline PBMC samples, isolated from blood collected before immunotherapy onset, using the PamChip peptide microarray system. The PamChip consists of identical arrays, each containing 144 unique protein tyrosine kinase phosphorylation sites. Kinase activity in PBMC protein lysates is measured in time and analyzed using BioNavigator software.

- Kinase activity profiles were generated by analyzing phosphorylation signatures of PBMC lysates on a peptide micro-array.
- The PamChip (PamGene, Netherlands) microarray comprises 144 different peptides derived from protein phosphorylation sites that are substrates for protein tyrosine kinases[1].
- Predictive models were trained using Partial Least Squares Discriminant Analysis (PLS-DA)[2] with log-transformed (anti-CTLA4) or VSN normalized (anti-PD1)[3] PamChip data. Predictive performance of the models was evaluated by estimating the Correct Classification rate (CCR) using cross-validation[2].
- Binary grouping in responder and non-responder to therapy (table 2) was based on RECIST v1.1[4].

	Responder	Non-Responder
Model 1: BOR	CR/PR/SD	PD
Model 2: PFS	Late/no progression	Early progression (<140 d)

Table 2. Responder and non-responder definitions based on BOR (model 1) and PFS (model 2).

Conclusions

- Kinase activity profiles of PBMC samples prior to checkpoint inhibitor therapy can predict the likelihood of response to anti-PD1 or anti-CTLA4 therapy.
- This assay may serve as a rapid and fast predictive liquid biomarker to stratify patients prior to treatment.
- Heparin collection tubes displays a stable kinase activity profile, whereas EDTA interferes with kinase activity over time.
- Results suggest the involvement of immune receptor kinases, underlying the mechanism of response to checkpoint inhibitor therapy.

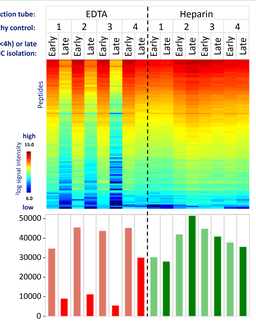

Figure 3. Late PBMC isolation from EDTA collection tubes, 24h after blood collection, results in a strong decrease in kinase activity compared to early PBMC isolation (<4h). For PBMCs isolated from Na-heparin collection tubes no effect on overall kinase activity is detected.

Results

	Treatment	Patients	Prediction CCR % (90%CI)
Cohort 1	Anti-CTLA4	N=10	100%(90%CI 74-100%)[1]
Cohort 2	Anti-CTLA4	N=29	83% (90%CI 67-93%)[1]
Cohort 3	Anti-PD1	N=33	70%(90%CI 54-83%)[1]
Cohort 4	Anti-PD1	N=33	70%(90%CI 54-83%)[2]
Cohort 5	Anti-PD1	N=38	75%(90%CI 59-85%)[1]

Table 3. Response classification based on RECIST v1.1: [1]model 1 or [2]model 2.

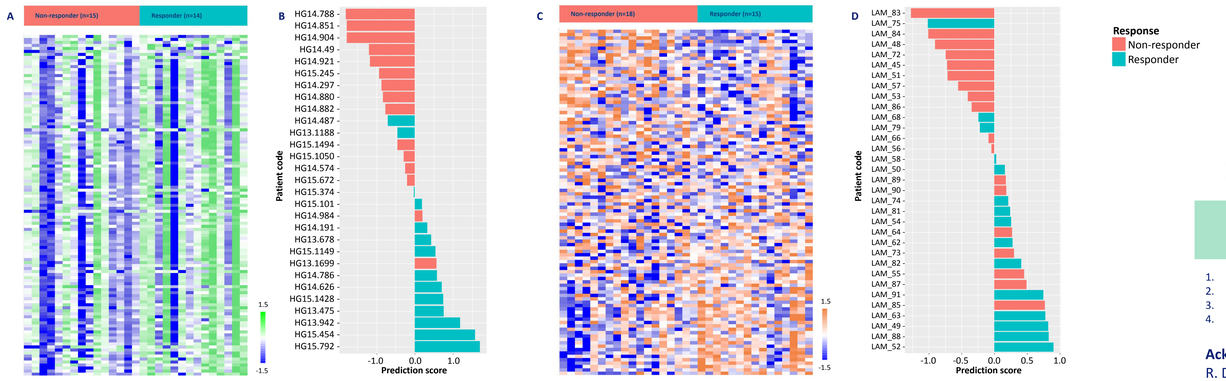

Figure 2. Data visualization showing the correlation between kinase activity profiles and treatment response for two examples from table 3. A: protein tyrosine kinase activity profiles for cohort 2 (anti-CTLA4) shown as a heatmap with relatively high activity shown in green relatively low activity in blue. B: PLS-DA Prediction scores obtained by cross-validation showing the performance of response prediction (non-responder vs. responder) for cohort 2. C: protein tyrosine kinase activity profiles for cohort 3 (anti-PD1) shown as a heatmap with relatively high activity shown in orange and relatively low activity in blue. D: PLS-DA Prediction scores obtained by cross-validation showing the performance of response prediction (non-responder vs. responder) for cohort 3.

References

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