Radboudumc

Signal transduction pathway activity of TGF-β and Hedgehog as possible response predictors to checkpoint inhibition in patients with advanced melanoma; a retrospective cohort study

Stefan G. van Ravensteijn[1], Daniele V.F. Tauriello[2], Avital Amir[3], Yvonne J.W. Wesseling-Rozendaal[4], Anne van Brussel[4], Diederick M. Keizer[4], Henk M.W. Verheul[1'5] and Kalijn F. Bol[1]

1: Department of Medical Oncology, Radboud university medical center, Nijmegen, the Netherlands; 2: Department of Medical Biosciences, Radboud university medical center, Nijmegen, the Netherlands;

3: Department of Pathology, Radboud university medical center, Nijmegen, the Netherlands; 4: InnoSIGN B.V. High Tech Campus 11, 5656AE, Eindhoven, the Netherlands; 5: Department of Medical Oncology, Erasmus Medical Center, Rotterdam, the Netherlands

Aim

To quantify signal transduction pathway (STP) and pSMAD2 activity in advanced melanoma and to characterize their predictive and prognostic role in treatment with immunotherapy.

Background

- Immunotherapy is highly effective in patients with metastatic melanoma, significanlty proloning progression-free survival (PFS) and overall survival (OS).
- However, a significant number of patients do not respond to treatment with immunotherapy.
- Unambiguous biomarkers predicting response to immunoterhapy are lacking.
- Specific features of the tumor microenvironment (TME) such as elevated TGF-β levels might be critical determinants of recurrence and metastasis.
- TGF-β pathway activity is hypothesized to prevent T-cells to enter the tumor and leading to immune escape.

Method

- 34 patients with metastatic melanoma, first line treatment with Nivolumab or Pembrolizumab.
- Metastatic tumor tissue (n=34) and paired primary tumor tissue (n=31) obtained before treatment with immunotherapy.
- Responders (n=15; partial or complete response) and non-responders (n=19; progression within 6 months).
- OncoSIGNal pathway activity profiling RT-qPCR test (InnoSIGN) to measure the activity of 8 different STPs (TGF-β, Hedgehog (HH), AR, PI3K, MAPK, Notch, NFkB and JAK-STAT1/2), expressed on scale of 0-100.
- STP activity will be correlated to treatment response, PFS, OS.
- Immunohistochemistry (IHC) staining investigating pSMAD2 expression (hallmark protein TGF-β pathway) by means of anti-pSMAD2 antibody (1:50, clone 138D4, #3108, Cell Signaling Technology). Correlated to response, PFS, and OS.
- pSMAD 2 IHC staining scored based on percentage of nuclei stained, and intensity.

Results

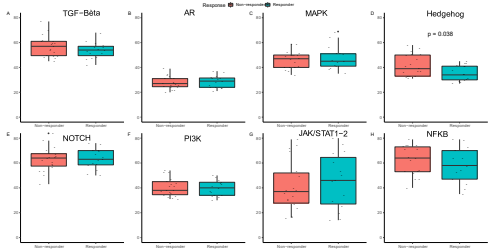

Figure 1: Box plots reflecting the individual STP scores in metastatic tumor samples comparing responders (n = 15) and non-responders (n = 19). T-tests were used to compare means.

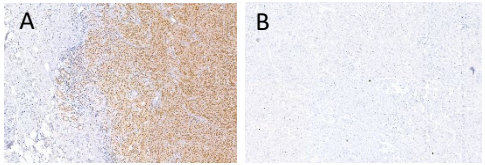

Figure 2: Nuclear immunohistochemical staining investigating pSMAD2 expression on melanoma tumor tissue. Samples were scored based on intensity and percentage of cells stained postive (A) or negative (B).

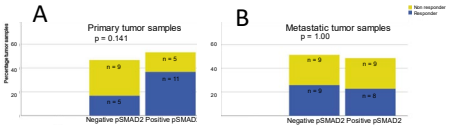

Figure 3: Bar chart reflecting the percentage of positive and negative IHC stained tumor samples for responders and non-responders. Primary tumor tissue (A) and metastatic tumor tissue samples (B) were stained. Fisher exact test was used.

Pathway [mean [range]]	Primary tumor tissue (n = 31) Responder (n = 14)	Non-responder (n = 17)	p-value	Metastatic tumor tissue (n= 34) Responder (n = 15)	Non-responder (n = 19)	p-value
TGF-β	50.86 [36-60]	50.00 [32-63]	0.751	53.87 [42-68]	56.84 [45-77]	**0.265**
HH	33.64 [24-49]	32.06 [19-48]	0.569	35.73 [27-45]	41.63 [31-58]	**0.038**
MAPK	55.71 [25-66]	56.53 [43-73]	0.810	47.47 [35-69]	46.21 [34-59]	0.664
AR	28.43 [20-37]	27.41 [20-35]	0.542	28.47 [21-37]	27.84 [20-39]	0.721
NOTCH	67.50 [52-85]	64.53 [53-74]	0.301	63.27 [50-76]	62.79 [43-84]	0.880
PI3K	37.71 [25-49]	38.59 [30-56]	0.741	39.60 [30-50]	40.26 [31-54]	0.784
JAK/STAT1-2	54.23 [20-77]	56.25 [28-84]	0.784	45.53 [14-80]	41.17 [16-79]	0.562
NFκB	65.92 [35-82]	69.45 [51-80]	0.476	58.15 [35-79]	61.94 [40-79]	0.441

Table 1: Mean STP scores measured in primary and metastatic melanoma tumor tissue for Responders and Non-Responders to immunotherapy. AR; Androgen Receptor, HH; Hedgehog. T-test were used to compare means.

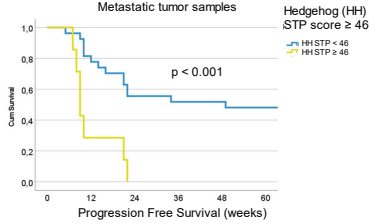

Figure 4: Kaplan-Meier estimate and log-rank test reflecting PFS in weeks comparing metastatic tumor samples with a Hedgehog STP score ≥ 46 (n=7) vs <46 (n=27).

Predictive and prognostic role of STP scores on treatment response

- Multivariable logistic regression analysis showed Hedgehog (HH) STP activation in **metastatic tumor samples** as the sole (negative) predictor associated with response (**OR 0.884 (95% CI 0.789-0.990), *p*=0.033**).
- Multivariate cox-regression analysis showed HH STP in **metastatic tumor samples** as the sole predictor associated with PFS (**HR 1.077 (95%CI 1.016-1.141), p=0.012**).
- Logistic- and cox-regression analysis revealed no significant differences based on TGF-β STP scores for response, PFS, and OS in primary and metastatic tumor samples.
- Logistic- and cox-regression analysis revealed no significant differences based on all STP scores in primary tumor samples regarding response, PFS, and OS for all investigated pathways.

Conclusions

- No correlation between TGF-β—whether determined by signal transduction pathway scores or by pSMAD2 IHC assessment—with treatment response to immunotherapy, PFS, and OS; in both primary tumor samples and metastatic tumor samples.
- Increased Hedgehog activity in metastatic tumor samples, but not in primary tumor samples, might be related to a decreased response and worse PFS from immunotherapy.

Author information

S. Van Ravensteijn, MD:
Stefan.Ravensteijn@radboudumc.nl
K.Bol, MD, PhD:
Kalijn.Bol@radboudumc.nl