(Neo-)adjuvant ipilimumab + nivolumab (IPI+NIVO) in palpable stage III melanoma: Updated data from the OpACIN trial and first immunological analyses

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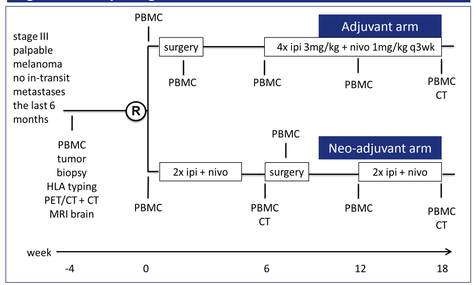
Background

- The outcome of high risk stage III melanoma is poor (20-59% 5-year survival). [1-3]
- Adjuvant CTLA-4 blockade (ipilimumab, 10mg/kg 4 cycles + 3 year maintenance) improves 5 year recurrence-free survival (RFS) and overall survival (OS).[4]
- Currently adjuvant PD-1 blockade is tested in phase 3 adjuvant trials.
- The combination of ipilimumab plus nivolumab has been shown to improve objective response rates (ORR), progression free survival (PFS) and OS as compared to ipilimumab (and ORR and PFS numerically as compared to nivolumab).[5]
- Neo-adjuvant treatment may be a favorable as immune checkpoint inhibition is of greatest value at the moment of TCR triggering and dependent on the amount of antigen present.
- Thus, we postulated that adjuvant immunotherapy will work most efficiently, when immunotherapy is initiated prior to surgery.

Methods

- Two-arm Phase 1b feasibility trial in 20 patients
- All patients received the combination of ipilimumab + nivolumab
- Randomized to either adjuvant, or split neo-adjuvant and adjuvant

Figure 1. Study design



Co-Primary Endpoints

- Safety and feasibility as measured by immune related adverse events (irAEs) and adherence to the timelines in the study protocol
- Alteration in magnitude or breadth of the neo-antigen specific T cell response in the time interval pre- to post-adjuvant therapy in peripheral blood.

Secondary Endpoints

- RFS, as determined according to RECIST 1.1
- Rate and type of late adverse events
- Correlation between RFS and the of magnitude and/or breadth of neo-antigen T cell population

Key eligibility criteria

- Histologically confirmed stage 3b metastatic cutaneous melanoma, palpable disease (no in-transit only) of the axilla or groin
- No prior immunotherapy targeting CTLA-4, PD-1 or PD-L1
- Normal LDH
- Adults at least 18 years of age
- World Health Organization (WHO) Performance Status 0 or 1
- Presence of at least two of the defined HLA alleles that allow MHC tetramer analysis

Current analysis

- Median follow-up post-surgery is 14 months (datalock May 2017)

Clinical Results

Table 1. Baseline characteristics

	Adjuvant (n=10)	Neo-adjuvant (n=10)
Mean age, years, +/-SD	51+/-7y	55+/-13y
Sex – male (n)	7	6
WHO 0 (n)	10	10
No of BL lymph nodes (median)	2 (1-7)	1 (1-5)
Stage IIIB/IIIC (No. of patients)	8/2	4/6
LDH normal (n)	10	10
CRP normal (n)	8	10
ALC normal (n)	10	10

Table 2. Treatment-related adverse events

Adverse event	All grades (n)	Grade 3/4 (n)
Any adverse event	20	18
Elevated ALT	17	4
Diarrhea	12	6
Elevated Lipase	11	8
Fatigue	10	0
Rash	10	4
Nausea	9	0
Colitis	7	6
Hyperthyroidism	7	1
Hypothyroidism	7	0
Vomiting	6	3
Headache	6	2
Fever	5	4
Weight loss	4	0
Adrenal Insufficiency	3	1

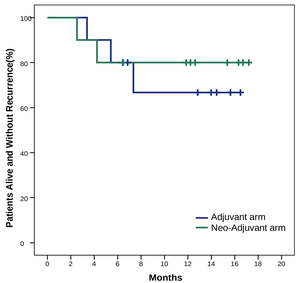
Safety and feasibility

- All patients in the neo-adjuvant arm have undergone lymph-node dissection on the pre-planned time-point.
- Only 2/20 patients received all four courses (17 patients stopped due to grade 2-4 toxicity and 1 due to PD after two courses adjuvant).
- No surgery-related adverse events were attributed to the immunotherapy.

Table 3. Response of neo-adjuvant treated patients

ID	Courses	Radiologic response (CT scans, mm)	Pathologic response	Total # muations
7	2	31 x 50 → 18 x 31	pCR	625
16	2	23 x 36 → 17 x 23 & 22 x 24 →9 x 12	pCR	44
19	2	24 x 40 → 19 x 24	pCR	
4	3	21 x 47 → 11 x 34	micrometastases (<1mm)	250
5	2	9 x 10 → ND	micrometastasis (0.5mm)	
8	2	10 x 12 → 6 x 9	micrometastasis (sporadic tumor cells)	59
14	4	18 x 19 & 25 x 37 → ND	micrometastasis (sporadic tumor cells)	373
24	2	28 x 40 → 15 x 21	macrometastasis (75% necrosis)	
13	2	22 x 40 → 22 x 40	LNs 35mm, 2mm, 1mm, 0.5mm, 0.1mm	135
17	1	11 x 18 → 17 x 25	LNs 30mm, 13mm, 6.0mm, 3.5mm	

Figure 2. Recurrence-free survival



Efficacy

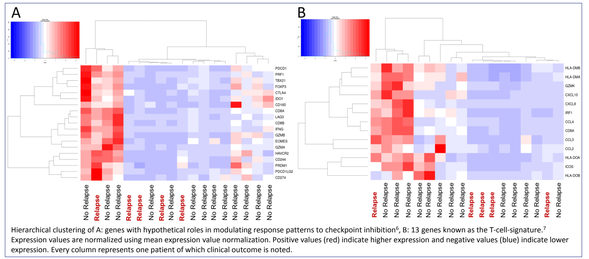
- Pathological response rate was 80% in the neo-adjuvant arm.
- 5 patients relapsed, all relapses were early after post-surgery (median of 4.2 months).
- 2/10 (20%) patients in the neo-adjuvant arm relapsed (SD, only 2 courses due to grade 3 colitis, PD only 1 course due to grade 3 dermatitis).
- 3/10 (30%) patients relapsed so far in the adjuvant arm (one had 3 courses and two had 2 courses, stopped due to colitis, hypophysitis, and colitis, respectively).

Patients outcome

- So far 9/20 (33%) patients recovered fully from irAEs, 11 patients have ongoing AEs (8 need only hormonal substitution, 3 have other ongoing irAEs: low-grade diarrhea, PNP, and rash + elevated ALT/AST).
- All patients are still alive; however two are progressive upon last line standard therapy.

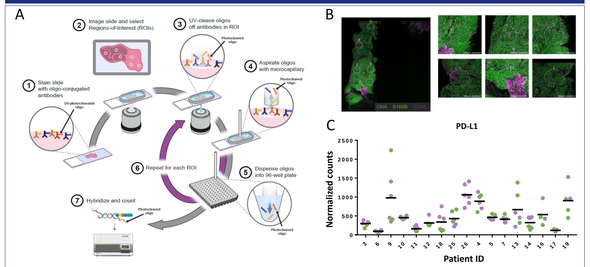
Immunological Analysis

Figure 3. RNA sequencing of baseline tumor biopsies



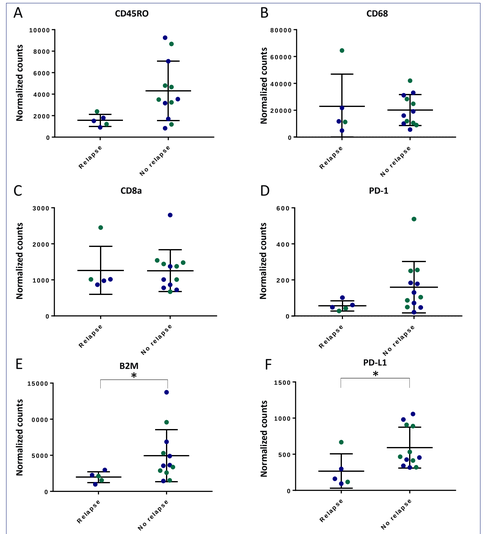
Hierarchical clustering of A: genes with hypothetical roles in modulating response patterns to checkpoint inhibition[6], B: 13 genes known as the T-cell-signature.[7] Expression values are normalized using mean expression value normalization. Positive values (red) indicate higher expression and negative values (blue) indicate lower expression. Every column represents one patient of which clinical outcome is noted.

Figure 4. Digital spatial protein profiling with the Nanostring™ microscope



A. Workflow of digital spatial protein profiling technique with the Nanostring microscope; B Example of ROI (region of interest) selection of 3 CD45 low and 3 CD45 high areas per tumor sample; C Overview of the different PD-L1 counts per patients per region (purple CD45hi ROI, green CD45lo ROI)

Figure 5. Baseline protein counts according to outcome



Baseline biopsies were stained with 26 antibodies and per patient 6 ROI (3 CD45hi and 3 CD45lo) were analyzed with the Nanostring microscope. Mean protein counts per patient (for A CD45 Ro, B: CD68, C: CD8a, D:PD-1, E: B2M and F: PD-L1) are displayed. Blue dots represents patients treated in the adjuvant arm, green dots represent patients treated in the neo-adjuvant arm. * P<0.05.

Conclusions

- Neo-adjuvant ipilimumab + nivolumab induces unexpected high frequency and depth of responses, but also a high percentage of grade 3 and 4 toxicities.
- At median follow up of 14 months none of the responders in the neo-adjuvant arm has relapsed.
- RNAseq based methods and mutational load do not seem to identify all patients with favorable outcome.
- Selective protein profiling (26 antibodies) of tumor (CD45lo) and margin areas (CD45hi) by the Nanostring™ microscope technique identified PD-L1 and B2M (absolute protein counts) as possible markers to identify patients benefitting from (neo)adjuvant ipilimumab + nivolumab; multi-parameter analysis might improve specificity.

References

[1]Balch et al., J Clin Oncol, 2009. [2]van Akkooi et al., Eur J Surg Oncol, 2007. [3]van der Ploeg et al., Ann Surg Oncol, 2011. [4]Eggermont et al., NEJM 2016, [5]Larkin et al., NEJM, 2015. [6]Hugo et al., Cell 2016, [7]Spranger et al., Nature 2015

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