Supplementary appendix

This appendix is a supplement to: Carvajal RD, Nathan P, Sacco JJ, et al. A phase 1 study of safety, tolerability, and efficacy of tebentafusp using a step-up dosing regimen and expansion in patients with metastatic uveal melanoma

Clinical trial registration number: NCT02570308

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Methods:

Pharmacokinetic (PK) and pharmacodynamic (PD) assessments were assessed from peripheral blood derived samples taken pre-treatment, after the first dose on Day 1 and after the third dose on Day 15. The concentration of tebentafusp in serum was measured by an electrochemiluminescent (ECL) immunoassay, utilizing biotinylated HLA-A2:gp100 antigen to capture tebentafusp via its TCR domain, a primary detection reagent of a rabbit anti-TCR polyclonal antibody, and a secondary anti-rabbit antibody labelled with SULFO-TAG. Luminescence was measured using the MSD Sector Imager 2400 (assay sensitivity 25 pg/mL) and PK profiles were analyzed using non-compartmental analysis (Certara Phoenix WNL version 8.2). Serum immune markers were analyzed using customized multiplex Luminex kits (Millipore) for the measurement of IFNγ, TNFα, IL-10, IL-2, IL-6, IL-1RA, CXCL10, CXCL9, CXCL11, CCL2, and HGF. Data was acquired and processed using xPONENT software on a Magpix Instrument (Luminex Corp.). Concentration of analytes in calibration standards, QC, and test samples was determined by interpolation of Median Fluorescence Intensity (MFI) values from standard curve fitted with 5-parameter logistic model.

Immunohistochemistry (IHC) analysis was performed on available baseline and on-treatment tumour biopsy samples. T cells were enumerated using primary antibodies against CD3 (clone 2GV6), CD4 (clone SP35) and CD8A (clone SP57), performed on the Ventana Discovery Ultra autostainer (Roche Diagnostics), following the manufacturer's instructions. Slides were visualized with a purple chromogen (Roche Diagnostics), counterstained with haematoxylin, dehydrated and cover slipped for viewing. Stained slides were scanned using the Pannoramic (3D Histech) whole slide scanner. Digital image analysis of the images was carried out using HALO™ software (Indica Labs), to quantify the number of positive cells within the tumour microenvironment.

Table A1. Treatment-emergent adverse events. Table summarizes treatment-emergent AEs that are present at least 10% any grade.

	Overall (N=42)	
	Any grade (≥ 10%)	Grade 3+
Preferred Term	**No. (%)**	**No. (%)**
Patients with any TEAE	42 (100%)	30 (71.4%)
Pyrexia	38 (90.5%)	2 (4.8%)
Nausea	31 (73.8%)	0
Fatigue	30 (71.4%)	4 (9.5%)
Pruritus	30 (71.4%)	2 (4.8%)
Chills	29 (69.0%)	0
Dry skin	27 (64.3%)	0
Oedema peripheral	27 (64.3%)	0
Periorbital oedema	24 (57.1%)	0
Hypotension	23 (54.8%)	5 (11.9%)
Vomiting	20 (47.6%)	1 (2.4%)
Abdominal pain	19 (45.2%)	5 (11.9%)
Back pain	19 (45.2%)	1 (2.4%)
Headache	19 (45.2%)	0
Arthralgia	18 (42.9%)	2 (4.8%)
Cough	18 (42.9%)	0
Erythema	17 (40.5%)	2 (4.8%)
Constipation	16 (38.1%)	0
Pain in extremity	16 (38.1%)	2 (4.8%)
Decreased appetite	15 (35.7%)	0
Diarrhoea	15 (35.7%)	1 (2.4%)
Generalised erythema	14 (33.3%)	2 (4.8%)
Hair colour changes	14 (33.3%)	2 (4.8%)
Rash	13 (31.0%)	2 (4.8%)
Myalgia	12 (28.6%)	0
Dizziness	11 (26.2%)	0
Dyspnoea	11 (26.2%)	1 (2.4%)
Influenza like illness	11 (26.2%)	0
Face oedema	10 (23.8%)	0
Musculoskeletal pain	10 (23.8%)	1 (2.4%)
Nasal congestion	10 (23.8%)	0
Skin mass	10 (23.8%)	0
Insomnia	9 (21.4%)	0
Rash generalised	9 (21.4%)	1 (2.4%)
Skin hyperpigmentation	9 (21.4%)	0
Skin hypopigmentation	9 (21.4%)	0

	Overall (N=42)	
	Any grade (≥ 10%)	Grade 3+
Preferred Term	**No. (%)**	**No. (%)**
Aspartate aminotransferase increased	8 (19.0%)	4 (9.5%)
Blood alkaline phosphatase increased	8 (19.0%)	2 (4.8%)
Hypophosphataemia	8 (19.0%)	6 (14.3%)
Pruritus generalised	8 (19.0%)	1 (2.4%)
Rash macular	8 (19.0%)	2 (4.8%)
Skin abrasion	8 (19.0%)	0
Urinary tract infection	8 (19.0%)	0
Alanine aminotransferase increased	7 (16.7%)	3 (7.1%)
Anaemia	7 (16.7%)	0
Dyspepsia	7 (16.7%)	1 (2.4%)
Hepatic pain	7 (16.7%)	0
Neck pain	7 (16.7%)	1 (2.4%)
Oropharyngeal pain	7 (16.7%)	0
Paraesthesia	7 (16.7%)	0
Peripheral sensory neuropathy	7 (16.7%)	0
Weight decreased	7 (16.7%)	0
Dermatitis acneiform	6 (14.3%)	1 (2.4%)
Gastrooesophageal reflux disease	6 (14.3%)	0
Haematoma	6 (14.3%)	0
Hyperbilirubinaemia	6 (14.3%)	2 (4.8%)
Malaise	6 (14.3%)	0
Rash maculo-papular	6 (14.3%)	1 (2.4%)
Upper respiratory tract infection	6 (14.3%)	0
Abdominal distension	5 (11.9%)	1 (2.4%)
Abdominal pain upper	5 (11.9%)	1 (2.4%)
Anxiety	5 (11.9%)	0
Hyperkalaemia	5 (11.9%)	0
Hypocalcaemia	5 (11.9%)	1 (2.4%)
Hypokalaemia	5 (11.9%)	0
Memory impairment	5 (11.9%)	0
Productive cough	5 (11.9%)	0
Rash pruritic	5 (11.9%)	0
Skin exfoliation	5 (11.9%)	0
Upper-airway cough syndrome	5 (11.9%)	0

Table A2: Composite terms for skin toxicity

Skin toxicity group	No. (%) of patients with event (N=42)
Rash	
Blister	3 (7.1)
Dermatitis acneiform	6 (14.3)
Dermatitis allergic	2 (4.8)
Dermatitis bullous	4 (9.5)
Palmar-plantar erythrodysaesthesia syndrome	3 (7.1)
Papule	2 (4.8)
Psoriasis	1 (2.4)
Rash	13 (31.0)
Rash erythematous	4 (9.5)
Rash generalised	9 (21.4)
Rash macular	8 (19.0)
Rash maculo-papular	6 (14.3)
Rash papular	3 (7.1)
Rash pruritic	5 (11.9)
Skin exfoliation	5 (11.9)
Pruritus	
Pain of skin	3 (7.1)
Pruritus	30 (71.4)
Pruritus generalised	8 (19.0)
Sensitive skin	1 (2.4)
Skin burning sensation	1 (2.4)
Pigment change	
Ephelides	2 (4.8)
Eyelash hypopigmentation	2 (4.8)
Hair colour changes	14 (33.3)
Skin hyperpigmentation	9 (21.4)
Skin hypopigmentation	9 (21.4)
Vitiligo	3 (7.1)
Erythema	
Erythema	17 (40.5)
Generalised erythema	14 (33.3)
Photosensitivity reaction	3 (7.1)
Dry skin	
Dry skin	27 (64.3)

Skin toxicity group	No. (%) of patients with event (N=42)
Edema	
Periorbital oedema	24 (57.1)
Skin tightness	2 (4.8)
Swelling face	1 (2.4)
Other changes	
Alopecia	4 (9.5)
Hyperhidrosis	3 (7.1)
Night sweats	3 (7.1)
Skin mass	10 (23.8)

Table A3. Estimated Cmax, AUC and t1/2 following first and third dose i.v. Infusion of tebentafusp. All patients received first dose of 20 mcg on cycle 1 day 1. Escalated dose amount on cycle 1 day 15 (and beyond) was cohort dependent. Data represent mean and [standard deviation (SD)]. 'NC', not calculated; [a] n=21.

Cohort		Cycle 1 Day 1 (mean [SD])			Cycle 1 Day 15 (mean [SD])		
		t1/2 (hr)	Cmax (pg/mL)	AUClast (day*pg/mL)	t1/2 (hr)	Cmax (pg/mL)	AUClast (day*pg/mL)
1 (n=3)	54 mcg	6.24	3070	1230	7.23	8980	3370
		[0.72]	[464]	[314]	[0.69]	[1540]	[1150]
2 (n=6)	64 mcg	7.96	3370	1440	8.07	9730	3940
		[1.92]	[764]	[334]	[1.69]	[2240]	[1270]
3 (n=4)	73 mcg	6.63	3090	1110	7.66	11400	4210
		[0.92]	[670]	[565]	[1.22]	[1330]	[385]
4 (n=6)	68 mcg	6.86	3720	1360	7.89	11800	4360
		[1.66]	[869]	[209]	[2.17]	[3060]	[918]
Expansion (n=23[a])	68 mcg	NC	4220	1840	NC	12700	5690
		[NC]	[1580]	[574]	[NC]	[4920]	[1900]

Table A4. Baseline and on-treatment biomarkers association with clinical response.

Biomarker	Baseline levels ≥ median vs < median		On-treatment change ≥ median vs < median fold change	
	TS Odds ratio (95% C.I.)	OS hazard ratio (95% C.I.)	TS Odds ratio (95% C.I.)	OS hazard ratio (95% C.I.)
CXCL11	2.1 (0.42,11.1)	1.9 (0.91,3.97)	3.5 (0.69,20.43)	1.3 (0.63,2.77)
IFNγ	3.5 (0.69,20.43)	2.7 (1.25,5.64)	3.5 (0.69,20.43)	1.9 (0.88,3.92)
IL-10	0.8 (0.16,3.85)	1.1 (0.54,2.29)	11.7 (1.92,100.84)	1.9 (0.9,3.99)
IL-1RA	1.3 (0.26,6.42)	1.4 (0.68,2.85)	3.5 (0.69,20.43)	1.2 (0.55,2.39)
HGF	2.8 (0.55,15.56)	2.7 (1.29,5.86)	2.1 (0.42,11.1)	0.9 (0.41,1.79)
CXCL9	3.5 (0.69,20.43)	1.6 (0.77,3.36)	0.8 (0.16,3.85)	0.9 (0.41,1.87)
IL-2	NA*	NA*	2.1 (0.42,11.1)	2 (0.92,4.16)
IL-6	2.1 (0.42,11.1)	2.8 (1.32,5.94)	3.5 (0.69,20.43)	1.4 (0.67,2.9)
CXCL10	1.3 (0.26,6.42)	1.4 (0.69,2.91)	1.3 (0.26,6.42)	0.7 (0.31,1.37)
CCL2	3.5 (0.69,20.43)	2.3 (1.09,5.03)	3.5 (0.69,20.43)	1.5 (0.7,3.11)
TNFα	1.3 (0.26,6.42)	1.3 (0.61,2.6)	2.1 (0.42,11.1)	2 (0.96,4.34)

*median was at the lower limit of quantification

Fig. A1. Progression Free Survival for tebentafusp-treated mUM patients. (A) Kaplan-Meier plot of progression free survival (N=42). Tick marks represent patients who were known to be alive and without disease progression as assessed per Response Evaluation Criteria in Solid Tumors, version 1.1, as assessed by investigators. The median (95% CI) progression free survival was 4.6 (1.9, 7.4) months.

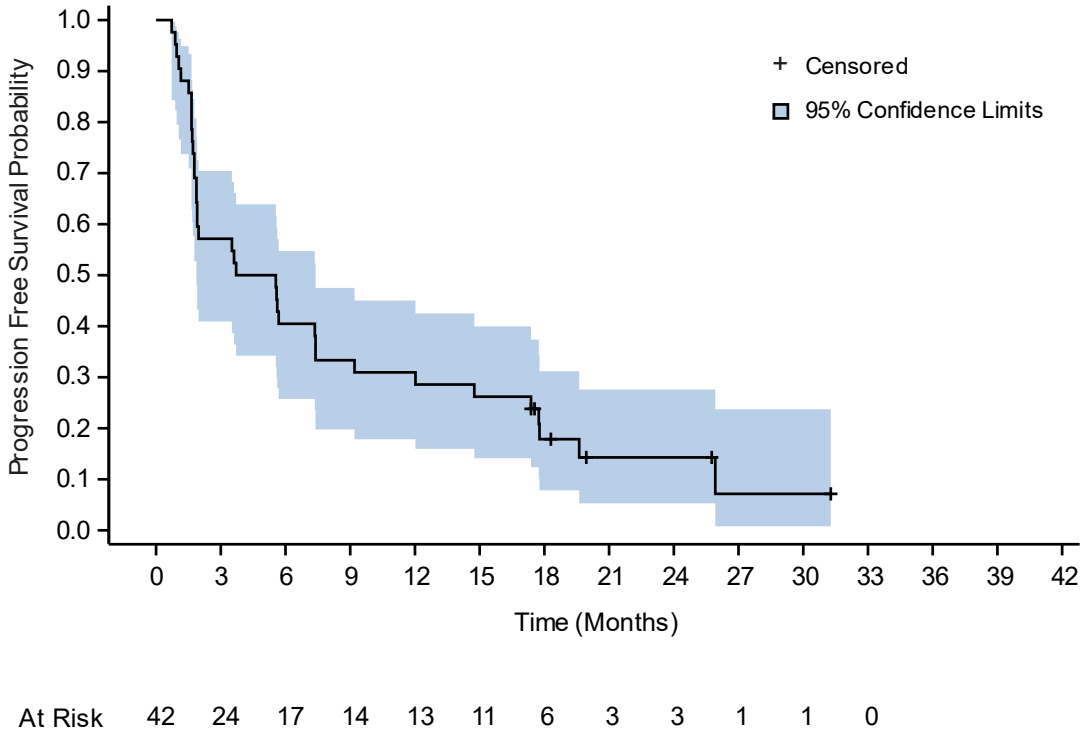
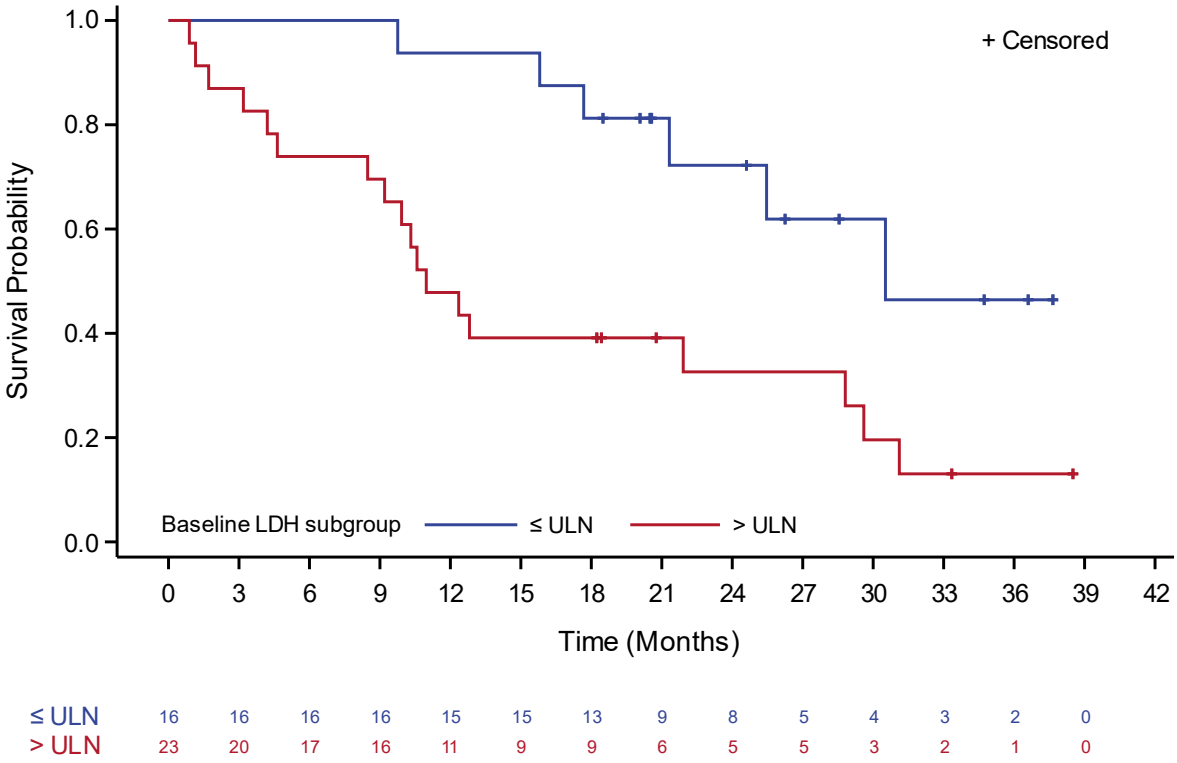


Fig. A2. Overall Survival by LDH status at baseline. Kaplan-Meier plot of overall survival by lactate dehydrogenase status (≤ ULN versus > ULN) at baseline (N=42). Events are deaths due to any cause. Patients not known to have died at the time of analysis are censored. Median (95% CI) OS was 30.5 (21.3, NR) months for patients with baseline LDH ≤ ULN and 11.0 (8.5, 28.8) for patients with baseline LDH > ULN. ULN, upper limit of normal



≤ ULN	16	16	16	16	15	15	13	9	8	5	4	3	2	0
> ULN	23	20	17	16	11	9	9	6	5	5	3	2	1	0

Fig. A3. Tebentafusp pharmacokinetics. Concentration: time plot for tebentafusp after (A) first dose on Cycle 1 Day 1 (20 mcg for all cohorts) and third dose on Cycle 1 Day 15 (Cohort 1, 54 mcg; cohort 2, 64 mcg; cohort 3, 73 mcg; Cohort 4 and Expansion subset, 68 mcg). Data points represent mean ± SD.

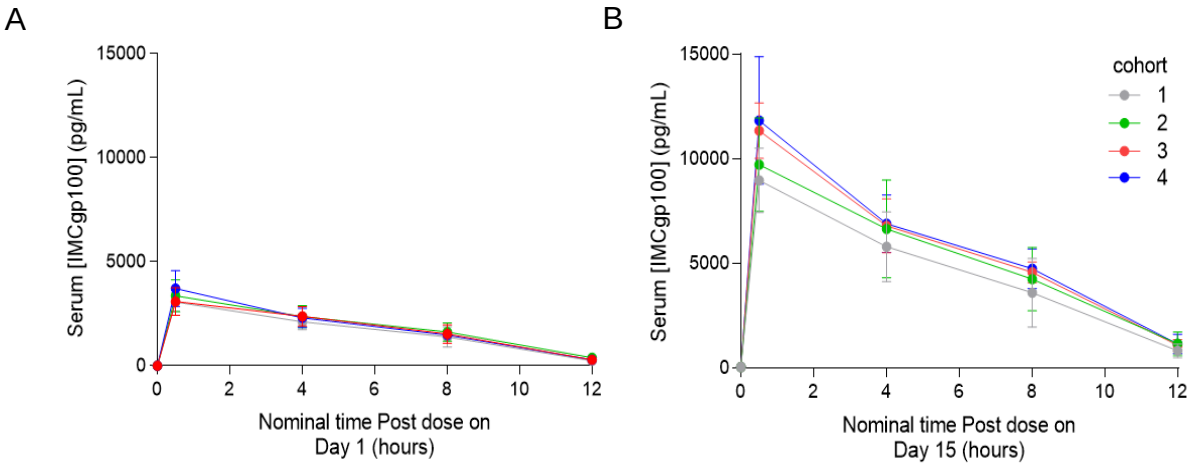


Fig. A4: Tebentafusp induced transient changes in serum cytokines by cohort. Temporal profile of serum cytokines post 1st and 3rd dose of tebentafusp expressed as fold-change relative to baseline (D1 Pre).

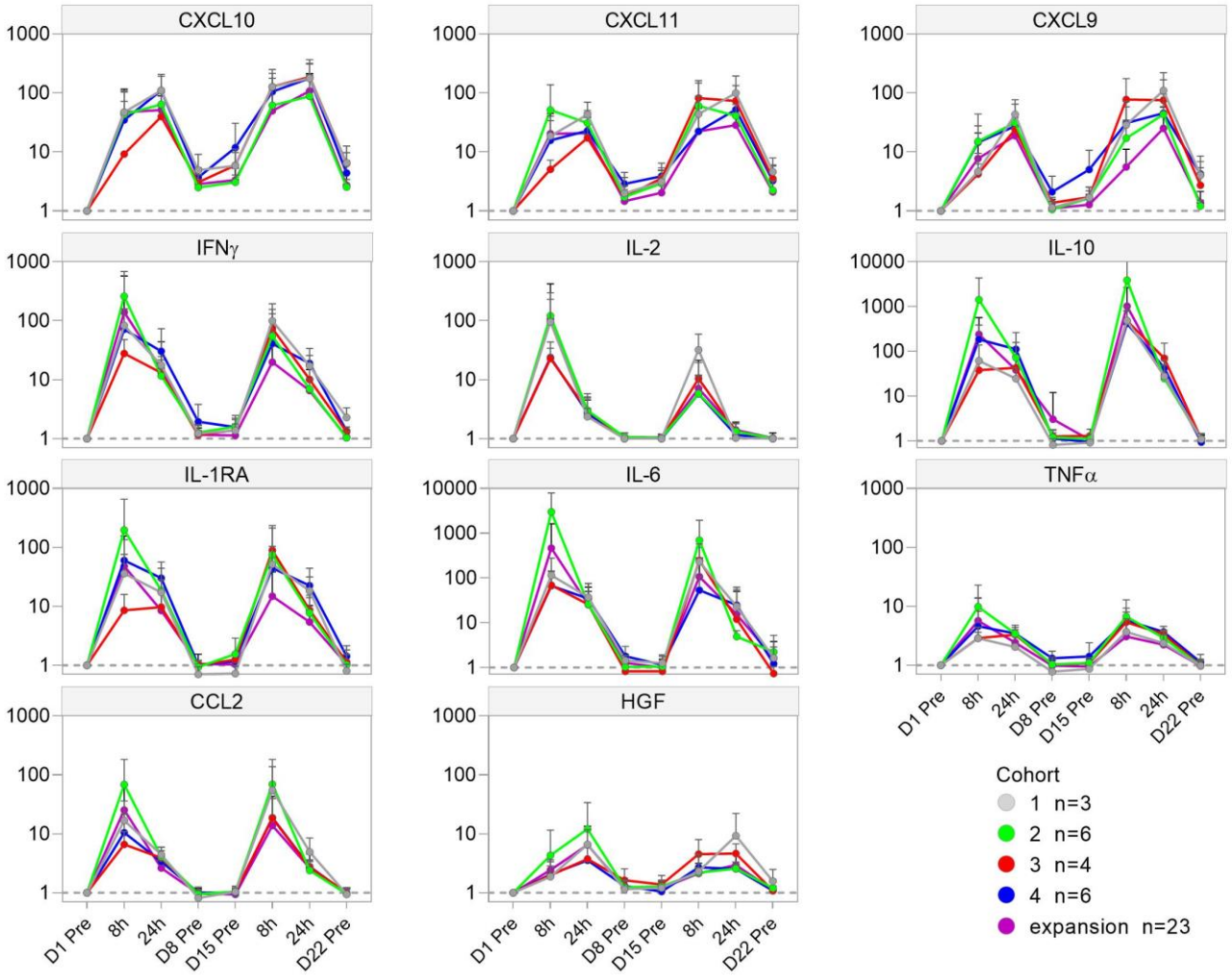


Fig. A5. Tebentafusp induced cytokine induction. Magnitude of fold-change in serum cytokines and chemokines 12-24h following first dose (D1) versus 3rd dose (D15); data points represent mean ± standard error of the mean [SEM], N=42.