Supplementary data

Platinum-based drugs disrupt STAT6-mediated suppression of immune responses against cancer in humans and mice

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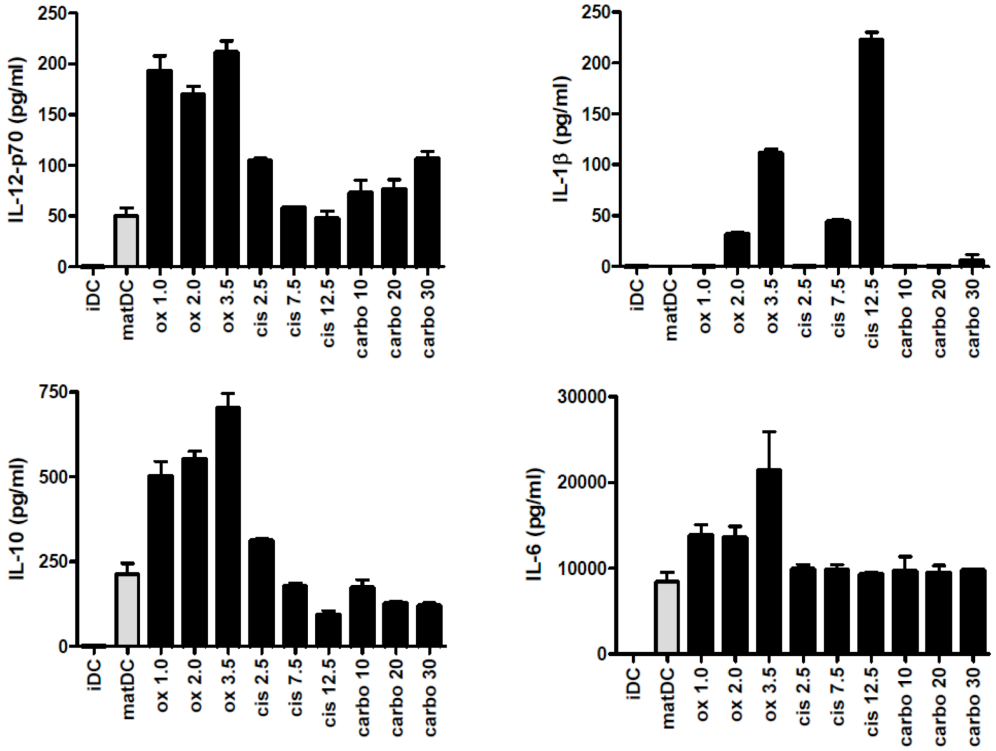
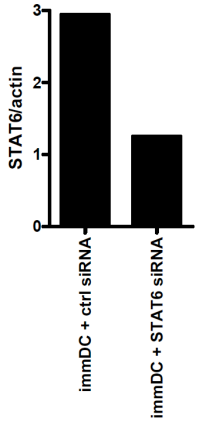
Supplementary figure 1



Supplementary figure 1. Cytokine production by monocyted-derived DCs, maturated by R848/Poly-I:C in the presence of clinically relevant

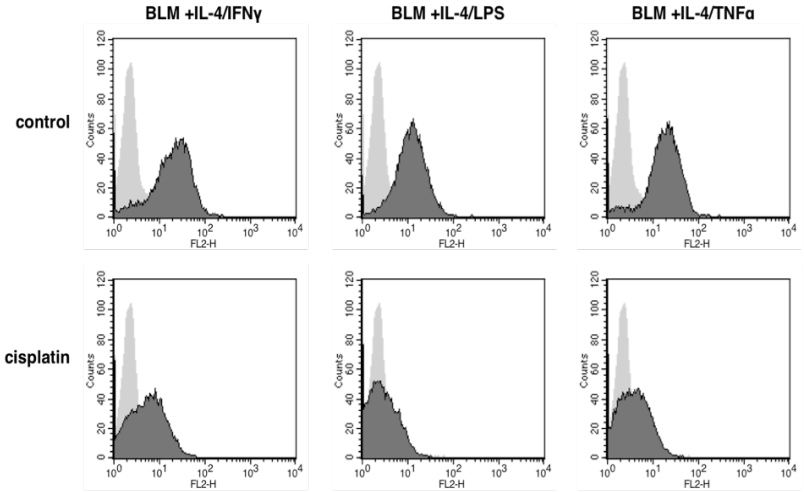
concentrations of platinum compounds (numbers on X-axis denote μg/ml). Levels are means with SD of 3 independent experiments.

Supplementary figure 2



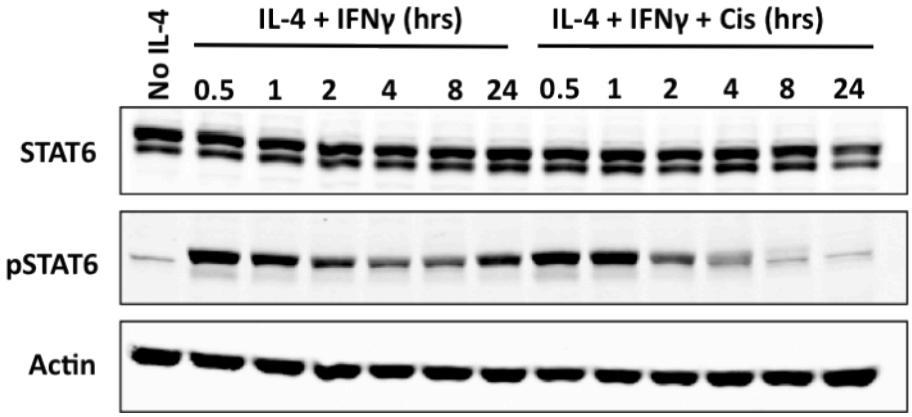
Supplementary figure 2. Knock-down efficiency of DCs transfected with control or STAT6-siRNA as measured by western blot analysis. The ratio of the integral intensity of STAT6 protein expression, normalized to the integral intensity of actin is shown.

Supplementary figure 3

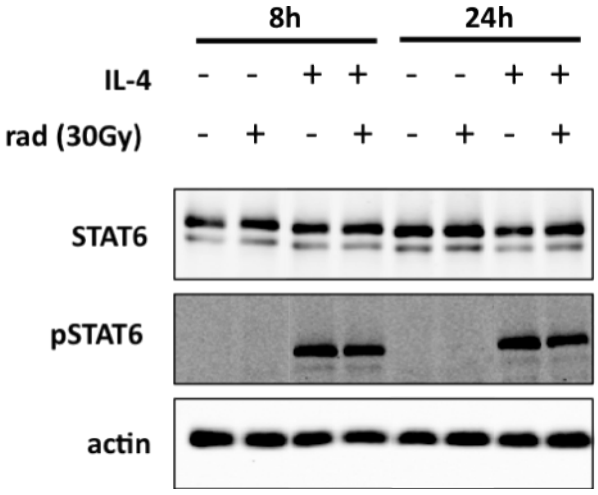


Supplementary figure 3. PD-L2 expression by BLM melanoma cell lines, treated with IL-4 combined with TNF-α/IFN-γ/LPS, with or without cisplatin 10 μg/ml (light-grey-isotype control, dark-grey PD-L2). A representative of 2 independent experiments is shown.

Supplementary figure 4



Supplementary figure 4. Western blot analysis of BLM melanoma cells. Expression of total STAT6, phosphorylated STAT6 and actin was measured 30 minutes, 1 hour, 2 hours, 4 hours, 8 hours and 24 hours after stimulation with IL-4/IFN-γ, with or without cisplatin 20 μg/ml. A representative of 2 independent experiments is shown.



Supplementary figure 5. Western blot analysis of BLM melanoma cells treated with or without radiotherapy. Expression of total STAT6, phosphorylated STAT6 and actin was measured 8 hours and 24 hours after stimulation with IL-4 or radiotherapy, as indicated. A representative of 2 independent experiments is shown.

Supplementary table 1. Characteristics of the chemotherapeutic agents used in the *in vitro* experiments.

compound	group	C_{max}*	clinical dose	reference	used dose *in vitro*
Methotrexate (Emthexate®, Pharmachemie, the Netherlands)	folic acid antagonist	363μg/ml	8-12g/m2	(1)	350μg/ml
Vincristine (Vincristine, Pharmachemie, the Netherlands)	vinca-alkaloid	0.37μg/ml	0.5mg/m^2	(2)	0.4μg/ml
Irinothecan (Campto®, Pfizer, Belgium)	Topo-isomerase I inhibitor	5μg/ml	350mg/m^2	(3)	5μg/ml
Etoposide (Toposin®, Pharmachemie, the Netherlands)	Topo-isomerase II inhibitor	15μg/ml	150mg/m^2 (oral)	(4)	15μg/ml
Bleomycin (Pharmachemie, the Netherlands)	anti-tumor antibiotic	0.19U/ml	30U	(5)	0.2U/ml
DTIC (Dacarbazin, Medac, Germany)	alkylating agent	150μg/ml	1900mg/m^2 (HD**)	(6)	150μg/ml
Cisplatinum (Platosin®, Pharmachemie, the Netherlands)	platinum	5 μg/ml	100mg/m^2	(7)	1; 2; 3; 5; 10 μg/ml
Oxaliplatin (Eloxatin®, Sanofi-Synhelabo, the Netherlands)	platinum	2.5-5.5μg/ml	130mg/m^2	(8)	0.5; 2; 3; 4; 5; 7μg/ml
Carboplatin (Carbosin®, Pharmachemie, the Netherlands)	platinum	40-80μg/ml	500mg/m^2	(9)	20; 40; 80; 120μg/ml
Cyclophosphamide (Endoxan®, Baxter, the Netherlands)	nitrogen mustard alkylating agent	140μg/ml	4g/m^2 (HD)	(10)	140μg/ml
Gemcitabine (Gemzar®, Eli Lilly, the Netherlands)	pyrimidine antagonist	23μg/ml	1000mg/m^2	(11)	25μg/ml
5-FU (5-Fluorouracil, Teva, the Netherlands)	pyrimidine antagonist	500μg/ml	1250mg/m^2 (capecitabine)	(12)	500μg/ml
Doxorubicine (Doxorubin®, Pharmachemie, the Netherlands)	anthracycline derivate	0.5μg/ml	30mg/m^2	(13)	0.5 μg/ml
Lenalidomide (Revlimid®, Cellgene-Europe, the Netherlands,)	Thalidomide analogue	0.5μg/ml	1x25mg	(14)	0.5μg/ml

*C_{max}: maximum concentration measured in blood of cancer patients after administration.
HD: high-dose regime

Supplementary table 2. Patient characteristics

	STAT6+	STAT6-
Cisplatin and Radiotherapy	**(n=37)**	**(n=21)**
median age (range)	55 (32-71)	57 (40-74)
Localization		
oral cavity/oropharynx	59%	47%
hypopharynx	41%	53%
T-stage		
T1	3%	5%
T2	11%	14%
T3	43%	43%
T4	43%	38%
N-stage		
N0	5%	14%
N1	11%	19%
N2	76%	62%
N3	8%	5%
Radiotherapy alone	**(n=24)**	**(n=24)**
Median age (range)	61 (46-78)	58 (41-76)
Localization		
oral cavity/oropharynx	71%	75%
hypopharynx	29%	25%
T-stage		
T1	0%	0%
T2	4%	8%
T3	54%	46%
T4	42%	46%
N-stage		
N0	0%	4%
N1	21%	21%
N2	75%	67%
N3	4%	9%

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