Anti-programmed-death-receptor-1 treatment with pembrolizumab in ipilimumab-refractory advanced melanoma: a randomised dose-comparison cohort of a phase 1 trial

Robert, C. et al. 2014 Lancet
Tolerance

• Tolerance - the absence of response to a specific antigen

• Also defined as “not doing harm to oneself”
  – Can have a response with no deleterious outcome

• Tolerance is antigen-specific: T and B cells
Autoimmunity: Loss of tolerance

- Normal individuals have self-reactive peripheral B and T cells
  - peripheral self-antigens that are not present during lymphocyte development and therefore do not initiate clonal deletion of specific lymphocytes

- Activation of self-reactive lymphocytes can lead to pathogenesis -> Autoimmunity
Tolerance occurs during lymphocyte development

• ‘Central’ tolerance
  • Clonal deletion (negative selection) to eliminate highly self-reactive lymphocytes
    – T cells: thymus
    – B cells: bone marrow
  • Clonal anergy - does not eliminate self-reactive lymphocytes but they become non-responsive
    – B cells: bone marrow
Central Tolerance is not enough?

• Antigens present in the thymus induce clonal selection
  – Self-proteins induce negative selection
    – self-proteins expressed in thymus
    – self-proteins in the circulation that get to the thymus
• TCR repertoire with high affinity for self is deleted

But…

• Naive peripheral T cells
  – Encounter self-proteins not seen in the thymus
  – Express TCRs with intrinsic affinity for self-MHC
  – Require survival signals via self-MHC plus peptide (low density or low affinity)
Common mechanisms of peripheral lymphocyte tolerance

- Anergy/hyporesponsiveness
- Ignorance
  - insufficient positive signals
  - dominant inhibitory signals
- Clonal deletion
- Immune Deviation
- Inhibition by specialized regulatory T cells
Presentation of self-proteins by BM-derived APCs vs MECs
The requirement for two signals for lymphocyte activation is critical for tolerance.

Increases in Signal 1 or Signal 2 can potentially lead to loss of tolerance.
Signal 2: COSTIMULATION

CTLA-4 binds to B7 with higher affinities than CD28

Biacore measurements, Collins et al. 2002 Immunity
Why two signals for activation?

• Lymphocytes specific for peripheral self-antigens due to lack of clonal deletion encounter tissue-specific antigens in the peripheral tissues
• Signal 2 is altered by changes in the microenvironment eg. inflammation
• Increased affinity and/or altered BCR specificity by somatic hypermutation during ongoing immune responses
CTLA-4 Control over Foxp3^+ Regulatory T Cell Function

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Fig. 1. Specific deletion of CTLA-4 expression in Foxp3^+ T cells results in fatal disease. (A) Flow cytometric analysis of intracellular Foxp3 (left) and CTLA-4 (right) in freshly isolated LN CD4^+ T cells from male FIC, CTLA-4^0/0, or BALB/c WT mice. (B) EGFP expression in CD4^+ or CD8^+ T cells derived from male FIC-CAG mice. (C) Sorted CD4^+EGFP^+ cells in FIC-CAG mice were stained for Foxp3. (D) CTLA-4 and Foxp3 expression in LN CD4^+ T cells from BALB/c WT, CKO, or KO mice. (E) Frequency of CTLA-4--expressing CD4^+Foxp3^+ T cells in CKO and normal littermates (n = 5). (F) Survival of KO and CKO mice as compared with normal littermates. Data represent three or more independent experiments. Vertical bars indicate SEM.

FIC foxp3-ires-cre
CAG Cre reporter

Science, 2008
CTLA-4 function

- Thymic selection?
- TCR repertoire shaping?
- Contraction of expansion

- Maintenance of naïve T cell tolerance and homeostasis
- Fail-safe rerouting of self-reactive T cells?

- Surface CTLA-4
- i.c. CTLA-4

Jain et al. PNAS 2010
Friedline et al. JEM 2009
Treg cells maintain naïve T cell quiescence via CTLA-4
Immune dysregulation in human subjects with heterozygous germline mutations in *CTLA4*

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Cytotoxic T lymphocyte antigen-4 (CTLA-4) is an inhibitory receptor found on immune cells. The consequences of mutations in *CTLA4* in humans are unknown. We identified germline heterozygous mutations in *CTLA4* in subjects with severe immune dysregulation from four unrelated families. Whereas *Ctla4* heterozygous mice have no obvious phenotype, human *CTLA4* haploinsufficiency caused dysregulation of FoxP3⁺ regulatory T (T<sub>reg</sub>) cells, hyperactivation of effector T cells, and lymphocytic infiltration of target organs. Patients also exhibited progressive loss of circulating B cells, associated with an increase of predominantly autoreactive CD21<sup>lo</sup> B cells and accumulation of B cells in nonlymphoid organs. Inherited human *CTLA4* haploinsufficiency demonstrates a critical quantitative role for CTLA-4 in governing T and B lymphocyte homeostasis.
Fig. 1. Clinical phenotype and pedigree of the patients. (A) Top: Computed tomography images of lung and brain from patient A.II.1. Bottom: Histological section (magnification 20×) from a duodenal biopsy from a healthy donor (HD) and patient A.II.1 stained for CD3 (brown cells), showing an increased number of transepithelial T cells within the villi. (B) Flow cytometric analyses of CD4⁺ cells or total lymphocytes stained for the indicated surface markers from a healthy donor and patient A.II.1. Data showing decreased CD45RA⁺CD62L⁺ naive CD4⁺ T cells are representative of three patients (A.II.1, A.II.1, and B.I.1). Programmed cell death-1 (PD1) expression data shown are representative of five patients (A.II.1, B.I.1, C.I.1, and D.II.1) and three healthy donors. Data showing decreased circulating B cells are representative of two patients (A.I.1 and A.II.1). (C) Mutations in patient alleles displayed on a schematic of the four exons of CTLA4, pedigrees, and phenotype summary highlighting organs (gray) with inflammatory infiltrates and autoimmune cytopenias for affected family members, TM, transmembrane domain. (D) Protein and mRNA expression of CTLA-4 in Treg cells. Left: Levels of CTLA-4 expression in Treg cells (CD4⁺CD25⁺FoxP3⁺) were assessed by intracellular staining. The numbers in the upper right corner depict mean fluorescence intensity (MFI) of anti-CD152 (CTLA-4) staining. Dotted line indicates the peak of CTLA-4 expression in a healthy donor. Data shown are representative of three experiments. Right: Levels of CTLA4 mRNA in Treg cells (CD4⁺CD25⁺CD127⁻) sorted from seven different healthy donors and four patients were measured by real-time PCR using the probe for CTLA4 transcript variant 1 (full length) and normalized to GAPDH. Data are means of replicates from six experiments. For relative gene expression, all data were normalized to the same HD. The horizontal lines indicate mean values from healthy donors or patients.

Fig. 2. Abnormal Treg cell phenotype and function in patients. (A) Flow cytometric analysis of FoxP3 and CD25 in CD4⁺ T cells from healthy donor (HD) and patients. (B) Mean fluorescence intensity of FoxP3 and CD25 in CD4⁺ FoxP3⁺ T cells from healthy donors and patients. Data are means ± SEM of replicates of indicated patient [A.I.1 (N = 7), A.II.1 (N = 1), B.I.1 (N = 2), C.II.1 (N = 2), D.II.1 (N = 4)] and 10 healthy donors. The N values represent number of
BTLA, B7-H4, B7x (tissues and tumors, not on APCs)

Modified from Freeman and Sharpe, 2003
PD-1, activation-induced analog inhibitor of T cell function

Okazaki…..Honjo, NRI 2013
Summary

**Background**
The anti-programmed-death-receptor-1 (PD-1) antibody pembrolizumab has shown potent antitumour activity at different doses and schedules in patients with melanoma. We compared the efficacy and safety of pembrolizumab at doses of 2 mg/kg and 10 mg/kg every 3 weeks in patients with ipilimumab-refractory advanced melanoma.

**Methods**
In an open-label, international, multicentre expansion cohort of a phase 1 trial, patients (aged ≥18 years) with advanced melanoma whose disease had progressed after at least two ipilimumab doses were randomly assigned with a computer-generated allocation schedule (1:1 final ratio) to intravenous pembrolizumab at 2 mg/kg every 3 weeks or 10 mg/kg every 3 weeks until disease progression, intolerable toxicity, or consent withdrawal. Primary endpoint was overall response rate (ORR) assessed with the Response Evaluation Criteria In Solid Tumors (RECIST, version 1.1) by independent central review.

**Findings**
173 patients received pembrolizumab 2 mg/kg (n=89) or 10 mg/kg (n=84). Median follow-up duration was 8 months. **ORR was 26% at both doses—21 of 81 patients in the 2 mg/kg group and 20 of 76 in the 10 mg/kg group (difference 0%, 95% CI −14 to 13; p=0.96).** Treatment was well tolerated, with similar safety profiles in the 2 mg/kg and 10 mg/kg groups and no drug-related deaths. The most common drug-related adverse events of any grade in the 2 mg/kg and 10 mg/kg groups were fatigue (29 [33%] vs 31 [37%]), pruritus (23 [26%] vs 16 [19%]), and rash (16 [18%] vs 15 [18%]). Grade 3 fatigue, reported in five (3%) patients in the 2 mg/kg pembrolizumab group, was the only drug-related grade 3 to 4 adverse event reported in more than one patient.

**Interpretation**
The results suggest that pembrolizumab at a dose of 2 mg/kg or 10 mg/kg every 3 weeks might be an effective treatment in patients for whom there are few effective treatment options.
“53% of patients had an objective response, all with tumor reduction of 80% or more”
Deletion of CTLA-4 on regulatory T cells during adulthood leads to resistance to autoimmunity

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