killer (NK) cells were reduced from 1153 to 958 live events, only a non-significant 17% reduction.

[008] Some studies have preconditioned patients with a chemotherapeutic agent, which was administered in combination with dexamethasone. For instance, Shi *et al* (Br J Haematol. 2008 Dec;143(5):641-53.) preconditioned relapsed multiple myeloma patients with fludarabine (Flu, 25 mg/m^2 on day −5 to day −2), and dexamethasone (Dex, 40 mg/d on days −5 to −2). This dose of dexamethasone corresponds to approximately 1.14 to 1.6 mg/kg within 72 hours prior to administration of ACT. Lymphodepletion was complete as shown in Figure 5 of Shi *et al* with absolute WBC reduced from 10.9e9/L to 0.7e9/L a 94% reduction. Shi *et al* did not suggest using higher dexamethasone doses. Cell Therapy Catapult has preconditioned patients with fludarabine x 5 days 30mg/m^2 intravenous (i.v.) and methylprednisolone x 1 day 500 mg i.v. for their A Phase I/II Study of Gene-modified WT1 TCR Therapy in MDS & AML Patients (https://clinicaltrials.gov/ct2/show/NCT02550535). This dose of methylprednisolone corresponds to a 100 mg dose of Dexamethasone which translates to about 1.4 mg/kg to 2 mg/kg. Additionally, both the Shi publication and the Cell Therapy Catapult trial preconditioned with repetitive doses of chemotherapy. Furthermore, methylprednisone increases Tregs, an undesireable response in the cancer patient where Tregs limit T cell tumor killing (Braitch Acta Neurol Scand. 2009 Apr; 119(4): 239–245; Mathian PLoS One. 2015; 10(12): e0143689).

[009] Because of the toxicities associated with chemotherapy or radiation and the contribution of the cytotoxic preconditioning to neuroedema and cytokine release syndrome after ACT, there is a need to develop safer and less cytotoxic methods to precondition patients to allow cellular immunotherapies to remain in the circulation or at the site of injection for extended periods of time.

Summary

[0010] The present inventors have shown the spleen accumulation of cellular immunotherapies to be a specific binding event, in contrast to the prior belief, discussed above, that spleen accumulation was due non-selective sequestration. The present inventors believe that prior art chemotherapy- and radiatiotherapy-based methods non-selectively destroy the cellularity of the spleen, to keep the administered cellular

immunotherapies in circulation or at the site of injection for extended durations of time and enhancing patient outcome.

[0011] The present patent application shows that non-chemotherapeutic agents, such as glucorticoids and other non-toxic lymphodepleting agents (NTLAs), can be used to make cellular immunotherapies more effective without the need for chemotherapy (or at least, can reduce the need for chemotherapy to just one day of chemotherapy treatment). For instance, the present application shows that acute doses of dexamethasone, typically about 300 to about 840 mgs, can be highly effective. In particular, the present disclosure discloses the benefit to cancer patients of pretreating the patient with a steroid such as dexamethasone shortly before cellular immunotherapy administration.

[0012] Thus, the present disclosure fills the need to replace chemo- and radiotherapy preconditioning by providing for methods and compositions for inhibiting binding of cellular immunotherapies to lymphoid tissue comprising administering cellular immunotherapies to an individual in conjunction with a therapeutic agent or agents that inhibit binding of cellular immunotherapies to lymphoid tissue, in particular to germinal centers and marginal zones in lymph nodes and germinal centers and marginal zones in the spleen. The therapeutic agent or agents also lymphodepletes peripheral blood lymphocytes via a biologic rather than a cytotoxic mechanism. The term "in conjunction with" can mean before, and/or together with, and/or after the cellular immunotherapies.

[0013] Accordingly, in a first example, this disclosure provides a method of enhancing adoptive cellular therapy (ACT) in a patient, by administering to the patient a non-toxic lymphodepleting agent (NTLA) at a dose that is effective to cause substantial lymphodepletion and/or cause ablation of secondary lymphatic germinal centers, wherein the method does not include the administration of radiotherapy nor a chemotherapeutic agent for a duration of more than 1 day within about 2 weeks preceding the start of ACT.

[0014] In a second example, this disclosure provides an NTLA for use in a method of enhancing adoptive cellular therapy (ACT) in a patient, the method comprising administering to the patient a dose of the NTLA that is effective to cause substantial lymphodepletion and/or cause ablation of secondary lymphatic germinal centers, wherein the method does not include the administration of a chemotherapeutic agent for a duration of 1 day or more.

[0015] In a third example, this disclosure provides a method of performing adoptive cellular therapy (ACT) in a patient in need thereof, said method comprising performing a method of enhancing ACT by pretreating the patient according to the disclosure; and then

performing the ACT by administering therapeutic cells to the patient.

[0015a] In one aspect, the present disclosure refers to use of a non-toxic lymphodepleting agent (NTLA) in the manufacture of a medicament for use in a method of enhancing adoptive cellular therapy (ACT) in a patient, wherein the NTLA is dexamethasone or another glucocorticoid, wherein the dexamethasone is to be administered to the patient at a human equivalent dose (HED) of at least about 3 mg/kg or another glucocorticoid is to be administered to the patient at an equivalent dose, wherein the method does not include the administration of a chemotherapeutic agent for a duration of 1 day or more.

[0015b] In another aspect, the present disclosure refers to use of a non-toxic lymphodepleting agent (NTLA) in the manufacture of a medicament for use in a method of performing adoptive cellular therapy (ACT) in a patient in need thereof, wherein an NTLA is to be administered to the patient according to any one of the preceding uses; and, the ACT is to be performed by administering the cells to the patient.

[0015c] In some examples, the NTLA is a steroid. The steroid may be a glucocorticoid. In some examples, the steroid is selected from the group consisting of dexamethasone, dexamethasone base, prednisone, methylprednisone, and dexamethasone analogues. In other examples, the NTLA is a selected from the group consisting of tacrolimus and cyclosporine.

[0016] In some examples, the dose of the NTLA achieves at least 60% CD3+ lymphodepletion. Preferably, the dose achieves at least 70%, at least 80% or at least 90% lymphodepletion. In preferred examples, the ACT involves administration of either a cell used to enhance the immune system in treating a disease in said patient or a cell derived from an immune lineage which directly treats said disease.

[0017] In examples in which the NTLA is dexamethasone, the dexamethasone may be administered at a dose of at least about 3 mg/kg, at least about 4 mg/kg, at least about 5 mg/kg, at least about 6 mg/kg, at least about 7 mg/kg, at least about 8 mg/kg, at least about 9 mg/kg, at least about 10 mg/kg, at least about 11 mg/kg, at least about 12 mg/kg, at least about 13 mg/kg, at least about 14 mg/kg, at least about 15 mg/kg, at least about 16 mg/kg, at least about 17 mg/kg, at least about 18 mg/kg, at least about 19 mg/kg, at least about 20 mg/kg, at least about 21 mg/kg, at least about 22 mg/kg, at least about 23 mg/kg, at least about 24 mg/kg, at least about 25 mg/kg, or at least about 26 mg/kg. The NTLA dexamethasone dose may be chosen from a range delimited by dexamethasone dosage values as disclosed herein, e.g. in the present paragraph. For instance the NTLA dexamethasone may be expressed as being administered at a dose chosen from a range of between about 9 mg/kg to about 12 mg/kg. In some examples, the dexamethasone may be administered at a dose of up to about 26 mg/kg.

[0018] Preferably, the NTLA is administered before ACT commences. The NTLA may be administered at least 12 hours before ACT commences. The NTLA may be administered at one or more time points between about 72 to about 12 hours prior to commencement of ACT.

[0019] In a fourth example, this disclosure provides a method of ameliorating the binding and accumulation of a cellular immunotherapy in secondary lymphatic binding sites comprising: identifying a patient suffering from cancer; administering to said patient a cellular immunotherapy comprising either a cell used to enhance the immune system in treating said cancer or a cell derived from an immune lineage which directly treats said cancer; and administering a non-toxic lymphodepleting agent (NTLA), which

lymphodepletes and ablates the secondary lymphatic binding sites where said cellular immunotherapy binds and accumulates, and is selected from the group consisting of: Tacrolimus delivered as an injection or oral dose of 0.48 mg/kg to about 10 mg/kg for about 1 - 4 weeks, Cyclosporine administered at about 15 - 100 mgs/kg/daily or about 7.5-50 mgs/kg/twice-daily for about 7 - 28 days, Dexamethasone base, or an equivalent dose of another glucocorticoid, between about 3 - 26 mg/kg for a single acute dose about 12-72 hours, and a TNF inhibitor administered for about 3 to about 4 weeks; wherein the administration of the NTLA occurs before administration said cellular immunotherapy, such as to ameliorate the binding and accumulation of said cellular immunotherapy in secondary lymphatic binding sites.

[0020] In preferred examples of the aspects described herein, the patient is a human. The NTLA may be administered at least 12 hours before ACT commences. In some examples, NTLA is administered within about 72 hours preceding commencement of ACT. In some examples, the ACT comprises administration of anticancer T cells and/or anticancer NK cells to the patient. In preferred examples, the cells administered for ACT include T cells. The ACT may be adoptive T cell therapy. Preferably, the method does not include the administration of radiotherapy or a chemotherapeutic agent for a duration of more than 1 day within about 2 weeks preceding the start of ACT. In some examples, no radiotherapy or chemotherapeutic agents are administered to the patient. In some examples, the NTLA induces an elevation of one or more plasma cytokine in the patient, selected from the group consisting of IL-2, IL-7, IL-12, and IL-15 to levels preferably of 20 pg/ml or greater. Levels of IL-6 may be unaffected by the NTLA.

[0021] The enhancement of ACT may comprise enhanced cancer killing in a cancer patient, or reduced autoimmune causing cell count in a patient with an autoimmune disorder, or reduced infectious agent load, in a patient with an infectious disease.

[0022] Preferably the NTLA is administered before ACT commences. For instance, NTLA may be administered at least 12 hours before ACT commences, or at another interval in advance of ACT, as described herein. In some examples, the NTLA is administered at one or more time points between about 12 to about 72 hours prior to commencement of ACT.

[0023] In some examples of this disclosure, the method according to any of the preceding claims, wherein the ACT is an adoptive T cell therapy, for instance based on

the transfusion of CAR T cells. In some examples, the patient is a cancer patient and the ACT comprises administration of anticancer T cells and/or anticancer NK cells.

[0024] The ACT may be based on autologous cells (i.e. cells that have been harvested from the patient, before being optionally modified or stimulated prior to transfer/readministration) or heterologous (i.e. cells or cell lines originating from another donor).

[0025] In some examples of this disclosure, no chemotherapeutic agents are administered to the patient.

[0026] A non-toxic lympodepleting therapeutic agent is an agent that induces lymphodepletion and inhibits germinal centers in the spleen without killing other rapidly dividing cells such as hair cells, mucosal cells, intestinal cells, and which does not induce sepsis, organ dysfunction, capillary leak, myocarditis, lethal inflammatory syndrome, fatal infusion reactions or subsequent new cancers.

[0027] The non-toxic lymphodepleting therapeutic agent can be administered without cytotoxic preconditioning or in combination with cytotoxic preconditioning. "In combination with" means administered before, and/or together with and/or after cytotoxic lymphodepleting chemotherapy, preferably with only one day of cytotoxic chemotherapy dosing. Addition of non-toxic lymphodepleting therapy can reduce the total dose or needed duration of cytotoxic lymphodepleting chemotherapy for lower adverse events and toxicities to the patient. The non-toxic lymphodepleting therapeutic agent can also be administered in combination with other cytotoxic preconditioning agents that include rituximab and similar molecules that bind lymphocytes and induce antibody-dependent cellular cytotoxicity (ADCC) and complement mediated cytotoxicity (CMC), in combination with temodar, in combination with interleukins or toll-like receptor agonists that can activate endogenous cytotoxic pathways, in combination with radiolabeled antibodies that trigger immune cell destruction (immunotoxins) such as antibodies to CD45, for instance Iomab-B, or in combination with total body radiation (TBI).

Brief Description of the Drawings

[0028] Figure 1: Acute high dose Dex eliminates ACT binding niches in the mouse spleen and secondary lymphatics. Black and white scale immunofluorescent pictures of fresh thick spleen sections stained with FITC-PNA to quantitate germinal centers from IP administered placebo control and mice IP administered HED 9.3 mg kg dexamethasone

Claims

1. Use of a non-toxic lymphodepleting agent (NTLA) in the manufacture of a medicament for use in a method of enhancing adoptive cellular therapy (ACT) in a patient, wherein the NTLA is dexamethasone or another glucocorticoid, wherein the dexamethasone is to be administered to the patient at a human equivalent dose (HED) of at least about 3 mg/kg or another glucocorticoid is to be administered to the patient at an equivalent dose, wherein the method does not include the administration of a chemotherapeutic agent for a duration of 1 day or more.

2. The use according to claim 1, wherein the ACT comprises either a cell used to enhance the immune system in treating a disease in said patient or a cell derived from an immune lineage which directly treats said disease.

3. The use according to claim 1 or claim 2, wherein the glucocorticoid is selected from the group consisting of dexamethasone base, prednisone, methylprednisone, and a dexamethasone analogue.

4. The use according to any one of the preceding claims, wherein the effective dose achieves at least 60% CD3+ lymphodepletion.

5. The use according to claim 3, wherein:

(i) the dexamethasone is to be administered at a human equivalent dose (HED) of at least about 4 mg/kg, at least about 5 mg/kg, at least about 6 mg/kg, at least about 7 mg/kg, at least about 8 mg/kg, at least about 9 mg/kg, at least about 10 mg/kg, at least about 11 mg/kg, or at least about 12 mg/kg;

(ii) the dexamethasone is to be administered at a human equivalent dose (HED) of up to about 26 mg/kg; or

(iii) the dexamethasone is to be administered at a human equivalent dose (HED) of between about 9 mg/kg to about 12 mg/kg.

6. The use according to any of the preceding claims, wherein the patient is a human.

7. The use according to any of the preceding claims, wherein the enhanced ACT comprises enhanced cancer killing, reduced autoimmune causing cell count, or reduced infectious agent load in a patient with a cancer, autoimmune or infectious disease, respectively.

8. The use according to any of the preceding claims, wherein the NTLA is:

(i) to be administered before ACT commences;

(ii) to be administered at least 12 hours before ACT commences; and / or

(iii) to be administered at one or more time points between about 72 to about 12 hours prior to commencement of ACT.

9. The use according to any of the preceding claims, wherein the ACT is adoptive T cell therapy.

10. The use according to any of the preceding claims, wherein no radiotherapy and no chemotherapeutic agents are to be administered to the patient.

11. The use according to any of the preceding claims, wherein the patient is a cancer patient and the ACT comprises administration of anticancer T cells and/or anticancer NK cells.

12. Use of a non-toxic lymphodepleting agent (NTLA) in the manufacture of a medicament for use in a method of performing adoptive cellular therapy (ACT) in a patient in need thereof, wherein an NTLA is to be administered to the patient according to any one of the preceding claims; and, the ACT is to be performed by administering the cells to the patient.

13. The use according to any one of the preceding claims, wherein the NTLA induces an elevation of one or more plasma cytokines in the patient, selected from the group consisting of IL-2, IL-7, IL-12, and IL-15 to levels preferably of about 20 pg/ml or greater.

14. The use according to any one of the preceding claims, wherein binding and accumulation of the cells in secondary lymphatic binding sites is ameliorated.