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Heterodimeric IL-15 treatment increases cytotoxic lymphocytes in LN follicles and reduces SHIV RNA

Dionysios C. Watson[1], Eirini Moysi[1], Antonio Valentin[1], Cristina Bergamaschi[2], Santhi Devasundaram[2], Sotirios P. Fortis[1], Claire Deleage[3], Jacob D. Estes[3], Elena Chertova[3], Jeffrey D. Lifson[3], Constantinos Petrovas[5], Barbara K. Felber[2], and George N. Pavlakis[1]

[1]Human Retrovirus Section, National Cancer Institute, Frederick; [2]Human Retrovirus Pathogenesis Section;Frederick; [3]AIDS and Cancer Virus Program, Leidos Biomedical Research, Inc., Frederick; [4]Biostatistics and Data Management Section, CCR, NCI, Rockville; and [5]Vaccine Research Center, NIAID, Bethesda

Background: Heterodimeric interleukin-15 (hetIL-15) is a native stable form of the cytokine that activates and expands cytotoxic T and NK cells. Based on its properties and extensive preclinical data, hetIL-15 is currently evaluated in humans for the treatment of cancer (NCT02452268). We study the effects of hetIL-15 in infected macaques to evaluate its use in HIV infection and especially in the reduction of SIV/SHIV reservoir towards a functional cure.

Methods: Rhesus macaques, either chronically infected by SHIV or uninfected received injections of hetIL-15 over 2 weeks using increasing doses of cytokine (step-dosing). At the end of the treatment, the animals were sacrificed and the hetIL-15 effects on different lymphocyte populations isolated from tissues collected at necropsy were monitored by multi-parametric flow cytometry and quantitative multiplexed confocal microscopy (histo-cytometry). Cell-associated viral RNA and plasma viral load was measured by quantitative PCR.

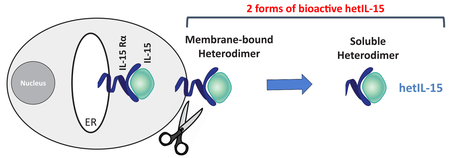
Results: This protocol was safe in rhesus macaques and resulted in systemic expansion of CD8+ T lymphocytes and NK cells with higher granzyme B content. These expanded cell populations were found in both effector sites, such as liver, vagina and rectum, and secondary lymphoid tissues. Importantly, a significant increase in cytotoxic effector memory CD8+ T cells was found in lymph nodes (LN) from all hetIL-15-treated macaques. CM9 tetramer staining demonstrated that the increase of CD8+ effector T cells in lymphoid organs included actively proliferating SIV-specific T cells with higher granzyme content. Imaging analysis by histo-cytometry revealed that these effector CD8+ T cells infiltrated the B cell follicles where chronically infected follicular helper CD4+ T cells are located. Following hetIL-15 treatment, cell-associated RNA was decreased in LN and plasma viral load was also decreased. Treatment of macaques under Antiretroviral Therapy (ART) with this regimen was also safe and induced cytotoxic CD8+ accumulation in LN follicles.

Conclusions: Step-dose administration of hetIL-15 is a well-tolerated regimen that results in systemic activation and expansion of cytotoxic leukocytes that infiltrate areas where chronic HIV-infected cells reside. These results suggest that hetIL-15 could be useful in disrupting sanctuary sites within the B cell follicles and reducing long-term viral reservoirs in HIV-1 infected individuals, thus contributing to a functional cure of the infection.

(published Watson, D.C., et al. PLoS Pathogen, Feb 2018)

http://journals.plos.org/plospathogens/article?id=10.1371/journal.ppat.1006902

IL-15 is a heterodimeric cytokine (hetIL-15)



Co-expression of IL-15 and IL-15Rα produces a stable heterodimer

- Natural way of cytokine production in vivo

Bergamaschi, J Biol Chem. 2008; J Immunol. 2009
Bergamaschi, Blood 2012; Chertova, J.Biol Chem 2013
Thaysen-Andersen Glycoconj J. 2016 33:417
Watson, Biomaterials 2016 105:195

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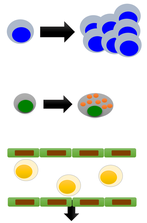
Frederick National Laboratory for Cancer Research, Frederick, MD [20878?]

Functions of hetIL-15



Lymphocyte Expansion and Differentiation

NK, CD8, CD4, γδ T cells

- Important for both Innate and Adoptive Immune System Homeostasis

Enhanced Activation, Cytotoxicity

Increases expression of granzymes, perforin
Enhances ADCC

Migration

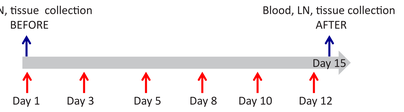
Rapid extravasation of lymphocytes to tissues

- Increases CD8 Effectors in tumors

hetIL-15 is A Unique Immune System Agonist For Cancer and AIDS Immunotherapy

2- or 3-week Administration of SC hetIL-15

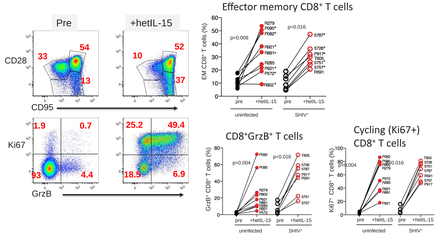
Similar to the clinical schedule



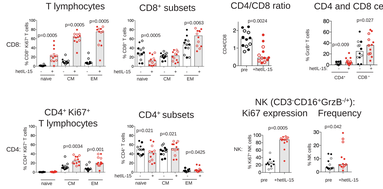
- High doses of hetIL-15 able to increase total body lymphocytes can be delivered without toxicity
- Important for combination of hetIL-15 with other drugs

Bergamaschi et al., Cytokine, 2018
https://www.ncbi.nlm.nih.gov/pubmed/29402721

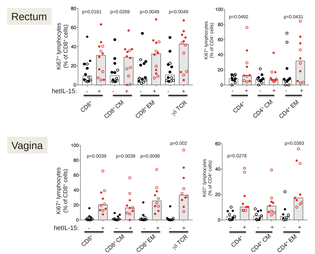
hetIL-15 Increases the Number of Cytotoxic Effector Lymphocytes and NK cells in Lymph Nodes



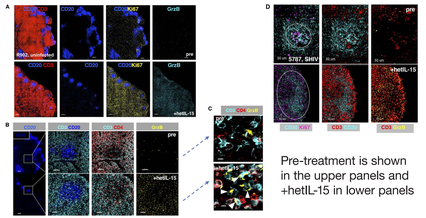
hetIL-15 Effects in Lymphocytes in Blood



hetIL-15 Effects in Mucosal Effector Sites



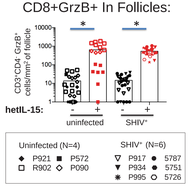
Accumulation of Ki67+ and GrzB+CD8+ T cells in Follicular Areas upon hetIL-15 Treatment



Pre-treatment is shown in the upper panels and +hetIL-15 in lower panels

(A) Confocal images showing the distribution of CD20 (blue), CD3 (red), Ki67 (yellow) and GrzB (cyan) positive cells in peripheral LN from an uninfected macaque (R902) before and after hetIL-15 treatment. Follicles are defined as CD20hi-dim areas.
(B) Higher magnifications from A of areas indicated by squares. CD20 (blue), CD3 (cyan), CD4 (red) and GrzB (yellow). Follicular areas defined by CD20 (left panel) show increased presence of CD8+ cells (defined as CD3+CD4-) (middle panel) and GrzB+ cells (right panel) upon hetIL-15 treatment. (C) Higher magnification of B shows presence of CD3+ CD4- GrzB+ (GrzB+CD8+) cells upon hetIL-15 treatment (white arrowheads). (D) Distribution of CD20 (blue), Ki67 (purple), CD3 (red) and GrzB (yellow) positive cells in a representative follicle. Pre (upper panels) and +hetIL-15 (lower panels) from an SHIV+ macaque (5787).

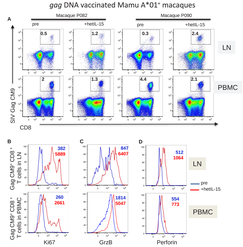
Histo-cytometry: Increased Infiltration of B Cell Follicles by GrzB+ CD8 Cells



Uninfected (N=4): P921, P572, R902, P090
SHIV+ (N=6): P917, 5787, P934, 5751, P995, 5726

Individual follicles were analyzed upon hetIL-15 treatment in LN from 4 uninfected and 6 SHIV+ macaques. A range of 2 to 14 follicles were analyzed/ animal. The numbers of $CD3^-CD4^-GrzB^+$ cells per mm^2 of area are in individual follicles for each animal are shown. Values of 0 were entered as 1 for the graph display only. Bars indicate average values. p values are calculated by two-way ANOVA for the SHIV+ versus uninfected and pre- versus post-hetIL-15 effects, with random effects to account for the clustered values by animal.

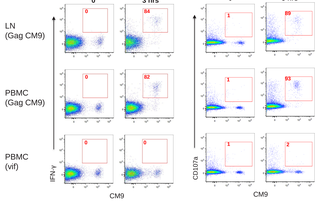
hetIL-15 Treatment Increases SIV-specific CD8+ T Cells in Peripheral Blood and LN



Dot plots showing changes in the frequency of Gag CM9-specific CD8+ T cells in axillary LN and PBMC upon hetIL-15 treatment of macaques P082 and P090. These animals received a SIV gag DNA vaccine. The CM9-tetramer responses are expressed as % of total T cells.

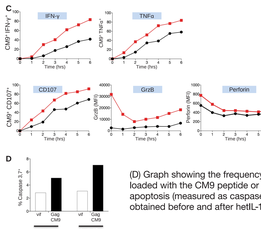
(B, C, D) Histogram overlays show increased levels of Ki67 (B), GrzB (C) and perforin (D) in Gag CM9-specific T cells upon hetIL-15 treatment in LN and PBMC. pre, blue line, +hetIL-15, red line. Numbers within the histogram overlays show the MFI for the specific markers.

Functional Assays for CM9-induced SIV-specific responses



Dot plots show production of IFN-γ (A) and degranulation CD107 (B) by CD8+ T cells specific for the MamuA01 restricted Gag CM9 epitope upon specific stimulation of the TCR with the CM9 peptide. The plots show the functional responses at 0 and 3 hours by lymphocytes from LNMC and PBMC of macaque P082. Similar data were obtained from macaque P090.

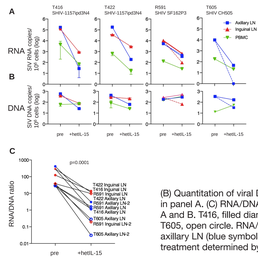
Functional Assays for CM9-induced SIV-specific responses (continued)



(C) Time course showing the frequency of IFN-γ, TNFα and CD107 positive $CD8^+$ $CM9^+$ T cells in blood, as well as changes in their content of granzyme B and perforin after stimulation with the CM9 peptide in lymphocytes recovered before (black symbols) and after (red symbols) *in vivo* hetIL-15 treatment. Results in (C) are from a separate experiment with similar results to that shown in panels A and B.

(D) Graph showing the frequency of bone marrow cells (macaque P090) loaded with the CM9 peptide or a peptide pool covering HIV-1 Vif undergoing apoptosis (measured as caspase 3 and 7+ cells) after incubation with LNMC obtained before and after hetIL-15 treatment.

hetIL-15 Treatment Reduces Viral RNA In LN



Quantitation of cell-associated viral RNA in axillary and inguinal LN and PBMC of 4 SHIV+ macaques upon hetIL-15 treatment.

The viral RNA determinations in T416 and T422 are from duplicate measurements from purified flash-frozen LNMC and PBMC. The viral RNA determinations in R591 and T605 are from single measurements of flash-frozen LN before treatment and from two independent flash-frozen LN collected after hetIL-15. Dotted and solid lines denote the two independent LN samples. No inguinal LN was available for T605 post treatment.

Axillary LN (blue square), inguinal LN (red triangle) and PBMC (green triangle) data are shown.

(B) Quantitation of viral DNA copies from the samples of the macaques in panel A. (C) RNA/DNA ratio of the measurements shown in panels A and B. T416, filled diamond; R591, open diamond; T422, filled circle; T605, open circle. RNA/DNA ratio are for inguinal LN (red symbols) and axillary LN (blue symbols). p value for the difference before and after treatment determined by Mann-Whitney test.

Changes in Plasma VL of 13 SHIV+ Macaques upon hetIL-15 Treatment

Macaque:	T413	T416	T419	T421	T422	T427	5726	5751	5757	5787	P917	R591	T605
SHIV:	1157	1157	1157	1157	1157	1157	327C	327C	327C	327C	SF162	SF162	CH505
Plasma VL:													
day 0, pre hetIL-15, week 1	4300	40	350	2300	3600	12	30	880	480	50	160	108	1950
week 2	1800	25	30	3500	1000	5	20	20	500	60	31	370	450
week 2 (necropsy)	1500	15	20	11000	230	3	10	5	5	7	3	140	120
VL fold drop, day 0 - week 2	3x	3x	18x	0.2x	16x	4x	3x	176x	96x	7x	53x	0.8x	16x

- Decrease in plasma VL upon hetIL-15 treatment in the majority of animals

Conclusions

hetIL-15 is a promising immune system agonist

- **Promotes a great infiltration of cytotoxic cells in B cell follicles and LN**
- Activates Lymphocytes (NK, CD8, CD4) and delivers to areas of tumor
- May provide a general method to enhance T-cell tumor entry, increasing the success rate of immunotherapy interventions?
- Homeostatic cytokine: Regulates number of lymphocytes
- Exogenous administration: Increases total body lymphocytes and replaces the need for lymphodepletion prior to adoptive cell transfer
- Elimination of the need for lymphodepletion could make more patients eligible for cell-transfer protocols

For AIDS functional cure

- **Cytotoxic T cells and NK entry into the follicle in massive numbers may enhance the elimination of virus expressing cells or may disrupt virus sanctuaries via altered cell interactions**