

UC-1V150, a potent TLR7 agonist capable of activating macrophages and potentiating mAb-mediated target cell deletion

Article

Accepted Version

Dahal, L. N., Gadd, A., Edwards, A. D. ORCID: <https://orcid.org/0000-0003-2369-989X>, Cragg, M. S. and Beers, S. A. (2018) UC-1V150, a potent TLR7 agonist capable of activating macrophages and potentiating mAb-mediated target cell deletion. *Scandinavian Journal of Immunology*, 87 (6). e12666. ISSN 0300-9475 doi: <https://doi.org/10.1111/sji.12666> Available at <https://centaur.reading.ac.uk/77191/>

It is advisable to refer to the publisher's version if you intend to cite from the work. See [Guidance on citing](#).

To link to this article DOI: <http://dx.doi.org/10.1111/sji.12666>

Publisher: Wiley

All outputs in CentAUR are protected by Intellectual Property Rights law, including copyright law. Copyright and IPR is retained by the creators or other copyright holders. Terms and conditions for use of this material are defined in the [End User Agreement](#).

www.reading.ac.uk/centaur

CentAUR

Central Archive at the University of Reading

Reading's research outputs online

UC-1V150, a potent TLR7 agonist capable of activating macrophages and potentiating mAb-mediated target cell deletion

Lekh N Dahal^{1*}, Adam Gadd², Alexander D Edwards³, Mark S Cragg¹ and Stephen A Beers^{1*}

1. Antibody & Vaccine Group, Cancer Sciences Unit, Faculty of Medicine, University of Southampton, Southampton General Hospital, Southampton SO16 6YD, UK
2. School of Biological Sciences, University of Reading, Whiteknights, Reading, RG6 6AD, UK
3. School of Pharmacy, University of Reading, Whiteknights, Reading, RG6 6AD, UK

Running title: UC-1V150 potentiates immunotherapy

Keywords: TLR, macrophage, Fc gamma Receptor, target cell deletion

* LND and SAB share joint senior authorship of this article

Address correspondence: Lekh N Dahal, L.N.Dahal@soton.ac.uk; Antibody & Vaccine Group, Cancer Sciences Unit, Faculty of Medicine, University of Southampton, Southampton General Hospital, Southampton SO16 6YD, UK

Funding: This work was supported by grants from Bloodwise (12050 to M.S. Cragg), and BBSRC (BB/F017189/1 to A.D. Edwards).

Abstract

Toll like receptors (TLR) are critical mediators of the immune system with their activation linked to infection, inflammation and the pathogenesis of immune diseases including autoimmunity and cancer. For this reason, over the last two decades, TLR and their associated signalling pathways have been targeted therapeutically to enhance innate and adaptive immunity. Several TLR ligands, both endogenous and synthetic are at various phases of clinical testing, and new ligands are continually emerging. Agonists of TLR7 are known immune response modifiers, simultaneously stimulating several cell types, resulting in immune cell activation and cytokine and chemokine release. The immune stimulating properties of the TLR7 agonist Imiquimod has also been exploited for use in the treatment of malignant superficial tumours of the skin. Here, we investigated a novel TLR7 agonist UC-1V150 and demonstrate it activates both human and mouse myeloid cells in vitro and in vivo, to deliver potent Fc γ R mediated engulfment of opsonised target cells.

Introduction

Toll like receptors (TLR) play a major role in host defence by sensing distinct molecular components of bacteria, fungi and viruses. Once engaged they elicit phenotypic and functional changes in the many immune cell populations that express these receptors, stimulating innate immunity ^{1,2}. TLR activation also supports the subsequent development of adaptive immunity and therefore TLR agonists can be harnessed to enhance vaccinal responses ³. Given their ability to provide these potent immune effects, TLR agonists are currently being explored in a number of pre-clinical and clinical immunotherapy studies, either as vaccine adjuvants or in combination with monoclonal antibodies (mAb) ⁴. However, very few TLR agonists have been approved for clinical use to date with a few notable exceptions: the *Mycobacterium bovis* bacillus calmette-guerin (BCG) and Imiquimod as monotherapies, and monophosphoryl lipid A (MPLA) as a vaccine component ⁵.

Imiquimod (Aldara®), is a TLR7 agonist which was developed as a topical cream and approved by the FDA in 2004 for the treatment of superficial basal cell carcinoma (BCC). Although its mechanism of action is not completely understood, it is thought to exert its anti-tumour effect by inducing apoptosis of the BCC cells ⁶. In a recent phase 2 study in patients with bladder cancer, Imiquimod showed clinical activity, with some complete responses ⁷. Imiquimod has also been examined in combination; decreasing the number of metastases in the B16F10 melanoma model in combination with radiotherapy; suggesting that Imiquimod could serve as a radiosensitizer in melanoma patients ⁸. However, topical application of Imiquimod can also induce and exacerbate autoimmune skin inflammation akin to psoriasis ⁹⁻¹². In the mouse these effects result from an influx of various immune cells into the skin, epidermal hyperplasia and Th-17/IL-23 activation. This has led to Imiquimod-induced mouse psoriasis being widely used as an animal model in which to study autoimmune skin inflammation ^{13, 14}. Although Imiquimod appears to be a viable option for the treatment of superficial skin malignancies, there is a pressing need to identify,

characterise and develop additional TLR agonists that provide more systemic and potent effects.

We recently examined the potential of a range of TLR agonists for their ability to activate innate immune cells, including mouse and human macrophages, in order to improve mAb-mediated immunotherapy¹⁵. Although Imiquimod demonstrated some activity, it was unable to significantly augment mAb-immunotherapy. One possibility was that Imiquimod is not sufficiently potent to elicit these responses and so here we examined the potential of an alternative TLR7 agonist, 4-[6-amino-8-hydroxy-2-(2-methoxyethoxy)purin-9-ylmethyl]benzaldehyde (UC-1V150)¹⁶. UC-1V150 is synthesized from 2,6-dechloropurine with a free aldehyde group on the benzyl moiety so that it can be coupled to auxiliary chemical entities/proteins of interest¹⁶ and has been successfully conjugated to mAbs of clinical relevance raising the prospect of cell-targeted TLR activation¹⁷. To date, the therapeutic effect of UC-1V150 has only been studied in the context of infectious disease, where it was seen to stimulate murine macrophages to produce the proinflammatory cytokines IL-6 and IL-12 in vitro and in vivo¹⁶. However, the ability of this reagent to provide adjuvant effects to mAb immunotherapy remains to be explored. Here we provide evidence that UC-1V150 potently activates macrophages, enhances engulfment of tumour cells in vitro, and augments mAb mediated target cell depletion in vivo.

Materials and Methods

Clinical Samples and Ethics

Ethical approval was obtained by Southampton University Hospitals NHS Trust from Southampton and South West Hampshire Research Ethics Committee. Informed consent was provided in accordance with the Declaration of Helsinki. CLL samples were from a Human Tissue Authority licensed University of Southampton, Cancer Sciences Unit Tissue Bank and leukocyte cones were from Southampton General Hospital National Blood Service.

Animals

Mice were bred and maintained in local facilities and experiments approved by the local ethical committee under Home Office license PPL30/2964. Experiments conformed to the Animal Scientific Procedure Act (UK).

Synthesis of 4-((6-amino-2-(2-methoxyethoxy)-8-oxo-7,8-dihydro-9H-purin-9-yl)methyl)benzaldehyde: UC-1V150

UC-1V150 was synthesized according to previously reported procedure¹⁶ and retrieved as an off-white solid, and reconstituted in DMSO.

Phagocytosis assay

PBMC from leukocyte cones were purified by LymphoprepTM (Axis-Shield) density centrifugation and cell number adjusted to a concentration of 1×10^7 cells/ml in RPMI 1640 conditioned with 1% Human AB serum, Penicillin, Streptomycin, L-Glutamine and Sodium pyruvate (Sigma-Aldrich), 2ml/well plated in a 6 well tissue culture plate and left to incubate for two hours at 37°C in 5% CO₂. Non-adherent cells were then removed and adherent cells incubated for 6 days in the presence of 100ng/ml recombinant human M-CSF (generated in house) in RPMI 1640 containing 10% FCS, penicillin, streptomycin, L-glutamine and sodium pyruvate. Cells were fed on alternative days¹⁸. Human monocyte derived macrophages (hMDM) generated this way were then polarised to M1 or M2 using LPS: 50 ng/ml, recombinant human (rh) IFN- γ : 2 ng/ml (Peprotech), or rhIL-4: 10 ng/ml (Peprotech), rhIL-13: 10 ng/ml (Peprotech), respectively. Alternatively, hMDM were stimulated with Imiquimod (Invivogen) or UC-1V150 at 1 μ g/ml for 48h. Phagocytosis assays were then performed as previously described¹⁸ with human chronic lymphocytic leukaemia (CLL) cells as targets. Phagocytic index was calculated by dividing the percentage of phagocytic macrophages under the test condition by the percentage of phagocytic macrophages seen in the unstimulated condition.

Phenotypic analysis of macrophages and calculation of Fc γ R activatory:inhibitory

(A:I) ratio

Human and murine Fc γ R staining was performed as described previously¹⁹. Fluorescently conjugated mAb were obtained from BD Biosciences, AbDSerotec, eBioscience or made in-house. hMDM were stained with anti-human CD40-Alexafluor (AF)488 (Clone ChiLob7/6), CD38-AF488 (Clone AT13/5), both in-house, and CD11b-PE (eBioscience). Murine splenocytes were stained with anti-mouse CD11b-PE, Ly6C-PerCpCy5.5, Ly6G-PECy7 (eBioscience), and F4/80-APC (AbD Serotec). Samples were acquired on a FACS calibur or canto II (BD Bioscience) and data analysed with FCS express (DeNovo Software). The Fc γ R activatory:inhibitory (A:I) ratio for hMDM was calculated as: MFI for Fc γ RI*Fc γ RIIA*Fc γ RIII/Fc γ RIIB for hMDM and Fc γ RI*Fc γ RIII*Fc γ RIV/Fc γ RII for murine macrophages) giving a value of x for NT macrophage. The results for each test condition were then divided by x so that unstimulated macrophages received a ratio of 1. Using this approach allows each Fc γ R to contribute to the A:I ratio, regardless of the fluorochromes with which the detecting antibodies are labelled.

In vivo B cell depletion assays

Depletion of adoptively transferred hCD20 Tg B cells was performed as described previously¹⁸. In brief, splenocytes from target (T) hCD20Tg and non-target (NT) wild-type mice were labelled with 5 μ M and 0.5 μ M CFSE (Invitrogen), respectively, mixed (1:1), and i.v injected into recipients (5-8 \times 10⁶ cells/mouse). UC-1V150 (1-10 μ g) was administered after 24 and 48h, followed by Ritm2a (25 μ g) or isotype control. Spleens were harvested 16-20h after Ritm2a administration, splenocytes stained with anti-mouse CD19-APC (eBioscience) and assessed for T:NT ratio.

Statistical analysis

Statistical analysis was performed using GraphPadPrism. To compare differences between the experimental groups, student's t tests were performed with Welch's correction; a *P*-value <0.05 was considered significant at the 95% confidence interval. Stars denote significance as follows: **p* < 0.05, ***p* < 0.01, ****p* < 0.001, and *****p* < 0.0001.

Results and discussion

Agonists of TLR7 have previously been shown to modulate macrophage activation: The TLR7/8 agonist R-848 was shown to upregulate the activating Fc γ R and downregulate the inhibitory Fc γ RIIB²⁰, whereas the TLR7 agonist Imiquimod has been shown to induce pro-inflammatory cytokine release²¹. However, its effects on macrophage phenotype, Fc γ R expression and function is not known. To assess this and compare the stimulatory potential of UC-1V150, we generated macrophages from human peripheral blood monocytes and measured their response to Imiquimod and UC-1V150 versus the archetypal M1/M2 stimuli; LPS/IFN- γ and IL-4/-13, by measuring CD40 and CD38 for pro-inflammatory activation and CD11b and DC-SIGN as markers for alternative activation. UC-1V150, but not Imiquimod, showed an inflammatory profile resembling LPS/IFN- γ stimulated macrophages with increases in CD40 and CD38, decreased CD11b, and no change in DC-SIGN (Fig 1).

In line with the inflammatory phenotypic profile observed (Figure 1), UC-1V150 showed an increase in the expression of activating Fc γ RIIA and Fc γ RIII with no change in expression of inhibitory Fc γ RIIB (Fig 2A) resulting in an increased A:I ratio (Fig 2B), greater to that achieved by LPS/IFN- γ stimulation. We did not observe such profound changes in the Fc γ R profile and A:I ratio with the Imiquimod, indicating UC-1V150 is a more potent compound.

The phenotypic and Fc γ R changes induced by UC-1V150 were indicative of pro-inflammatory activation and therefore we assessed if these macrophages also displayed augmented functional activity in terms of their ability to phagocytose mAb-opsonised primary CLL cells (Fig. 2C) by flow cytometry. We have previously demonstrated, through a variety of methods, including fluorescence imaging and confocal microscopy techniques^{18, 28}, that phagocytic activity registered by flow cytometry is an accurate portrayal of macrophage engulfment of target cells rather than conjugate formation/rosetting around macrophages. Using this methodology Imiquimod treated macrophages from the majority of donors showed only a modest increase in the phagocytosis of target CLL cells, but UC-1V150 induced a

more robust ~1.5 fold increase in phagocytic index compared to untreated hMDM (Fig 2D), raising the possibility of synergistic combination with antibody therapeutics. Whilst data are presented for a single dose/concentration, prior reports^{24,25} suggest that this concentration is significantly above the ED50 for both compounds, and for UC-1V150 relatively selective for TLR7¹⁷. These compounds do not show major quantitative differences in potency when compared in concentration-response analysis of cellular activation or cytokine induction. Furthermore, we initially conducted a dose response (0.1, 1 and 10µg/ml of UC-1V150 in hMDM cultures and observed 0.1µg/ml had minimal effect on macrophage activation, whilst 10µg/ml was toxic to the cells [Data not shown].

After establishing the ability of UC-1V150 to modulate human macrophages in vitro both phenotypically and functionally, we asked whether such changes could be seen in vivo, in mice. We first injected mice intraperitoneally with UC-1V150 and investigated the differences in FcγR expression and A:I ratio induced on splenic macrophages. Without inducing a significant influx of inflammatory myeloid cells into the spleen (Fig 3A), UC-1V150 enhanced the levels of FcγRI and -IV on splenic macrophages (Fig 3B), culminating in an almost 4-fold increase in A:I ratio (Fig 3C). Using a robust, well established adoptive transfer model¹⁸, we then assessed the ability of UC-1V150 to augment the mAb-mediated depletion of transferred hCD20 Tg B cells. mAb-mediated depletion of hCD20 Tg B cells was also significantly enhanced by UC-1V150, both in the spleen (Fig 3D and E) and blood of recipient mice (Fig 3F).

Here we provide evidence for the ability of UC-1V150, a relatively new class of TLR7 agonist that has not been investigated in the context of mAb therapy and macrophage modulation, to augment mAb activity both in vitro and in vivo. Clearly, this has two important implications. First, repolarisation of macrophages to an inflammatory profile is a keenly sought goal for immunotherapy and the identification of cellular pathways and candidate drugs capable of reversing immune suppression in vivo, leading to macrophage polarisation, will allow

effective manipulation of these immune effectors in disease settings. This is important in the context of macrophages, as they can express all Fc γ R and have great potential to destroy tumours through Fc γ R-mediated phagocytosis¹⁸. Second, we provide evidence that this process is mediated by Fc γ R changes such that the A:I ratio is tipped in favour of optimal mAb therapy. Fc γ R play a critical role in regulating immune homeostasis and their expression must be carefully modulated such that the Fc γ R A:I ratio is appropriate, preventing unsolicited immune effects whilst permitting beneficial proinflammatory responses to ensue²². No overarching role has been ascribed to a single Fc γ R in mediating these effector activities, but it has previously been reported that high Fc γ RIIB expression may explain resistance to the anti-CD20 mAb rituximab; and that the composite function of the A:I ratio mirrors the efficacy of the TLR reagent in providing adjuvant effects to mAb immunotherapy²³. So-called “Danger signals” provided by TLR activation enable effective Fc γ R A:I ratio skewing, but their efficacy in vivo is limited. For instance, the TLR1/2 agonist Pam3CSK4 robustly induced favourable Fc γ R A:I changes, inflammation and phagocytosis of target cells in vitro, but failed to achieve this in vivo¹⁵. Therefore, drugs such as UC-1V150 that promote appropriate Fc γ R expression profiles in vivo, with the potential for murine to human translation raise the possibility of providing synergistic treatments with existing antibody therapeutics. However, TLR7 activation on B cells may influence selection, proliferation and diversification of the repertoire of B cells in the germinal centre, impeding the B cell tolerance checkpoints^{26, 27}. Therefore, a better understanding of the interaction between the B-cell receptor (BCR) and TLR7, as well as careful modulation of their responses, is critical when identifying potential therapeutic reagents targeting TLR7 as its hyperactivation may lead to autoantibody production by BCR activated cells potentially resulting in autoimmunity.

Figure legend

Figure 1: TLR-7 agonist UC-1V150 skews human macrophage towards LPS/IFN- γ like profile: hMDM were stimulated with LPS/IFN- γ (L/ γ), IL-4/-13 (4/13) or TLR7 agonists Imiquimod (Imi) or UC-1V150 for 48 hours and were assessed for changes in expression of cell surface markers CD40, CD38, CD11b and DC-SIGN by flow cytometry; **(A)** representative histograms. Open black histogram: Iso ctrl for non-treated (NT) macrophage, Filled black histogram: Expression of surface markers on NT macrophage, Open grey histogram: Iso ctrl for TLR7a treated macrophage, Filled grey histogram: Expression of surface marker in TLR7a treated macrophage. **(B)** MFI for each surface marker shown, data combined for 3-4 different donors, error bars \pm SEM (* compared to NT samples).

Figure 2: Fc γ R expression and antibody dependent phagocytosis of tumour cells following stimulation of hMDM with UC-1V150. A. hMDM stimulated with LPS/IFN- γ (L/ γ) IL-4/-13 (4/13), Imiquimod (Imi) or UC-1V150 for 48 hours were assessed for changes in expression of Fc γ R by flow cytometry; representative histograms shown. Open black histogram: Isotype control for non-treated (NT) macrophage, Filled black histogram: Expression of Fc γ R in NT macrophage, Open grey histogram: Isotype control for TLRa treated macrophage, Filled grey histogram: Expression of Fc γ R in TLRa treated macrophage. **B.** Changes in Fc γ R A:I ratio in relation to NT macrophages expressed as 1, data combined for 3-4 different donors, error bars \pm SEM, **C.** CFSE labelled target CLL cells opsonised with anti-CD20 antibody rituximab were co-cultured with hMDM previously stimulated with LPS/IFN- γ , IL-4/-13, or TLR7a and then assessed for phagocytosis. CFSE⁺CD16⁺ events indicate macrophages that have engulfed target cells. **D.** Phagocytic index calculated in relation to NT macrophages expressed as 1. Each data point represents an individual donor (* compared to NT samples).

Figure 3: Effect of UC-1V150 on mAb-mediated target B cell depletion in vivo. A-C. WT C57BL/6 mice were primed with UC-1V150 and the myeloid cells assessed in the spleen 48 hours later by flow cytometry. **A)** Cellular content: F4/80⁺ cells correspond to macrophages while Ly6C⁺ and Ly6G⁺ cells within the CD11b⁺ gate are monocytes and neutrophils, respectively. Upper dot plots: Naïve mice; lower dot plots: UC-1V150 treated mice. **B.** Expression of Fc γ R on splenic macrophages: open black histogram: Isotype control from naïve mice, filled black histogram: expression of Fc γ R from naïve mice; open grey histogram: isotype control from UC-1V150 treated mice, filled grey histogram: expression of Fc γ R from UC-1V150 treated mice. **C.** A:I ratio on splenic macrophages calculated from B. **D-E.** WT C57BL/6 were primed with UC-1V150 after transfer of target (T) hCD20Tg B cells

and non-Tg non-target (NT) B cells; and treated with Ritm2a. Mice were then assessed for target hCD20Tg B cell depletion in the spleen (**D, E**) and blood (**F**) and expressed as a T:NT ratio. Results shown are pooled from 2 independent experiments (1 experiment for blood) with n=3/group in each experiment).

References

1. Gordon S, Martinez FO. Alternative activation of macrophages: mechanism and functions. *Immunity* 2010; 32:593-604.
2. Xue J, Schmidt SV, Sander J, Draffehn A, Krebs W, Quester I, et al. Transcriptome-based network analysis reveals a spectrum model of human macrophage activation. *Immunity* 2014; 40:274-88.
3. Steinhagen F, Kinjo T, Bode C, Klinman DM. TLR-based immune adjuvants. *Vaccine* 2011; 29:3341-55.
4. Cheadle EJ, Lipowska-Bhalla G, Dovedi SJ, Fagnano E, Klein C. A TLR7 agonist enhances the antitumor efficacy of obinutuzumab in murine lymphoma models via NK cells and CD4 T cells. 2017.
5. Lu H. TLR Agonists for Cancer Immunotherapy: Tipping the Balance between the Immune Stimulatory and Inhibitory Effects. *Front Immunol* 2014; 5:83.
6. Oldfield V, Keating GM, Perry CM. Imiquimod: in superficial basal cell carcinoma. *Am J Clin Dermatol* 2005; 6:195-200; discussion 1-2.
7. Donin NM, Chamie K, Lenis AT, Pantuck AJ, Reddy M, Kivlin D, et al. A phase 2 study of TMX-101, intravesical imiquimod, for the treatment of carcinoma in situ bladder cancer. *Urol Oncol* 2017; 35:39.e1-.e7.
8. Cho JH, Lee HJ, Ko HJ, Yoon BI, Choe J, Kim KC, et al. The TLR7 agonist imiquimod induces anti-cancer effects via autophagic cell death and enhances anti-tumoral and systemic immunity during radiotherapy for melanoma. *Oncotarget* 2017; 8:24932-48.
9. Fanti PA, Dika E, Vaccari S, Miscial C, Varotti C. Generalized psoriasis induced by topical treatment of actinic keratosis with imiquimod. *Int J Dermatol* 2006; 45:1464-5.
10. Gilliet M, Conrad C, Geiges M, Cozzio A, Thurlimann W, Burg G, et al. Psoriasis triggered by toll-like receptor 7 agonist imiquimod in the presence of dermal plasmacytoid dendritic cell precursors. *Arch Dermatol* 2004; 140:1490-5.
11. Rajan N, Langtry JA. Generalized exacerbation of psoriasis associated with imiquimod cream treatment of superficial basal cell carcinomas. *Clin Exp Dermatol* 2006; 31:140-1.
12. Wu JK, Siller G, Strutton G. Psoriasis induced by topical imiquimod. *Australas J Dermatol* 2004; 45:47-50.
13. Flutter B, Nestle FO. TLRs to cytokines: mechanistic insights from the imiquimod mouse model of psoriasis. *Eur J Immunol* 2013; 43:3138-46.
14. van der Fits L, Mourits S, Voerman JS, Kant M, Boon L, Laman JD, et al. Imiquimod-induced psoriasis-like skin inflammation in mice is mediated via the IL-23/IL-17 axis. *J Immunol* 2009; 182:5836-45.
15. Dahal LN, Dou L, Hussain K, Liu R, Earley A, Cox KL, et al. STING activation reverses lymphoma-mediated resistance to antibody immunotherapy. *Cancer Res* 2017.
16. Wu CC, Hayashi T, Takabayashi K, Sabet M, Smee DF, Guiney DD, et al. Immunotherapeutic activity of a conjugate of a Toll-like receptor 7 ligand. *Proc Natl Acad Sci U S A* 2007; 104:3990-5.
17. Gadd AJ, Greco F, Cobb AJ, Edwards AD. Targeted Activation of Toll-Like Receptors: Conjugation of a Toll-Like Receptor 7 Agonist to a Monoclonal Antibody Maintains Antigen Binding and Specificity. *Bioconjug Chem* 2015; 26:1743-52.
18. Beers SA, French RR, Chan HT, Lim SH, Jarrett TC, Vidal RM, et al. Antigenic modulation limits the efficacy of anti-CD20 antibodies: implications for antibody selection. *Blood* 2010; 115:5191-201.
19. Tutt AL, James S, Laversin SA, Tipton TR, Ashton-Key M, French RR, et al. Development and Characterization of Monoclonal Antibodies Specific for Mouse and Human Fcγ Receptors. 2015; 195:5503-16.

20. Butchar JP, Mehta P, Justiniano SE, Guenterberg KD, Kondadasula SV, Mo X, et al. Reciprocal regulation of activating and inhibitory Fc{gamma} receptors by TLR7/8 activation: implications for tumor immunotherapy. *Clin Cancer Res* 2010; 16:2065-75.
21. De Meyer I, Martinet W, Schrijvers DM, Timmermans JP, Bult H, De Meyer GR. Toll-like receptor 7 stimulation by imiquimod induces macrophage autophagy and inflammation in atherosclerotic plaques. *Basic Res Cardiol* 2012; 107:269.
22. Dahal LN, Roghanian A, Beers SA, Cragg MS. FcgammaR requirements leading to successful immunotherapy. *Immunol Rev* 2015; 268:104-22.
23. Roghanian A, Teige I, Martensson L, Cox KL, Kovacek M, Ljungars A, et al. Antagonistic human FcgammaRIIB (CD32B) antibodies have anti-tumor activity and overcome resistance to antibody therapy in vivo. *Cancer Cell* 2015; 27:473-88.
24. Russo C, Cornella-Taracido I, Galli-Stampino L, Jain R, Harrington E, Isome Y, et al. Small molecule Toll-like receptor 7 agonists localize to the MHC class II loading compartment of human plasmacytoid dendritic cells. *Blood* 2011; 117: 5683-91.
25. Isobe Y, Tobe M, Ogita H, Kurimoto A, Ogino T, Kawakami H, et al. Synthesis and structure-activity relationships of 2-substituted-8-hydroxyadenine derivatives as orally available interferon inducers without emetic side effects, *Bioorg Med Chem* 2003; 11: 3641-7.
26. Boneparth A, Huang W, Bethunaickan R, Woods M, Sahu R, Arora S, et al. TLR7 influences germinal center selection in murine SLE. *PLoS One* 2015; doi: 10.1371
27. Kuraoka M, Snowden PB, Nojima T, Verkoczy L, Haynes BF, Kitamura D, Kelsoe G. BCR and Endosomal TLR Signals Synergize to Increase AID Expression and Establish Central B Cell Tolerance. *Cell Reports* 2017; 18: 1627-35.
28. Cleary KLS, HTC Chan, James S, Glennie MJ, Cragg MS, Antibody distance from the cell membrane regulates antibody effector mechanisms. *Journal of Immunology* 2017; 198: 3999-4011.

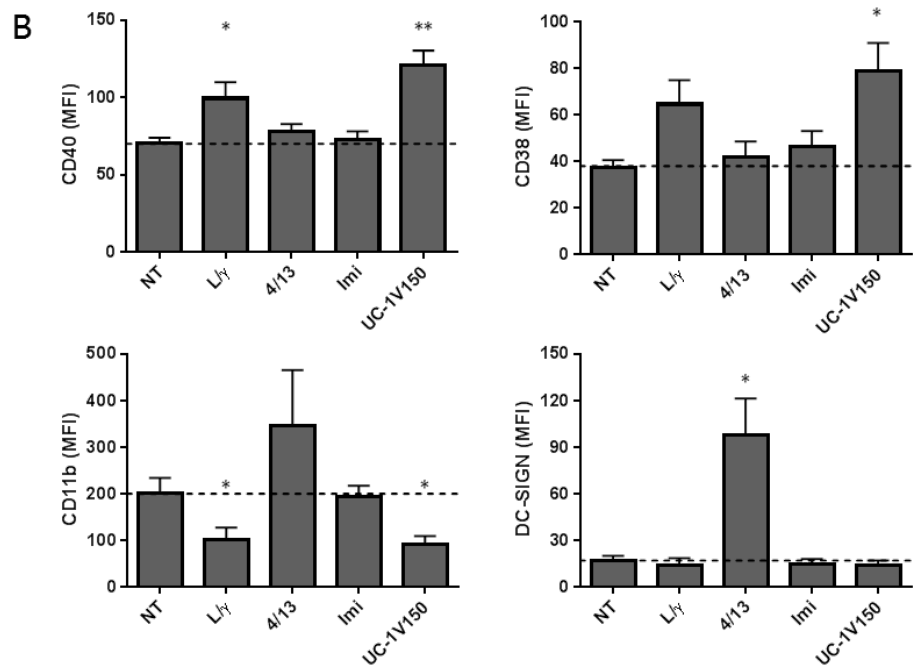
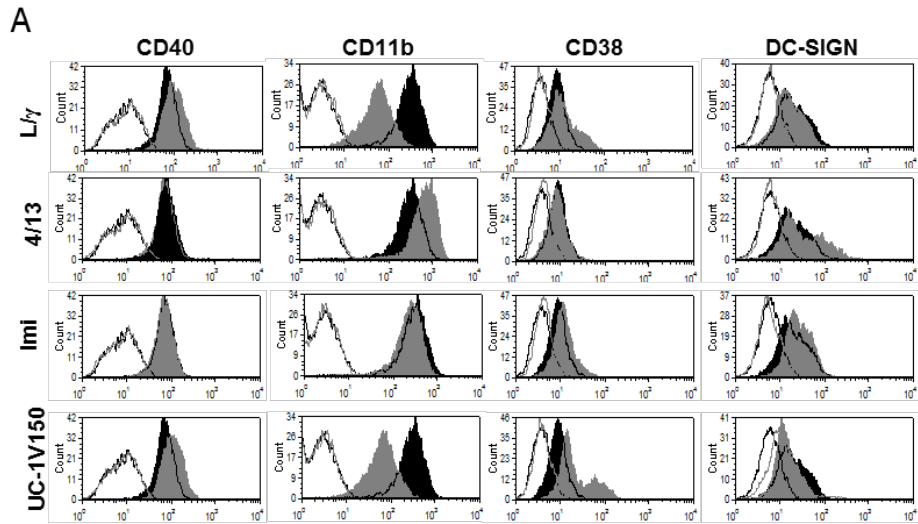


Figure 1

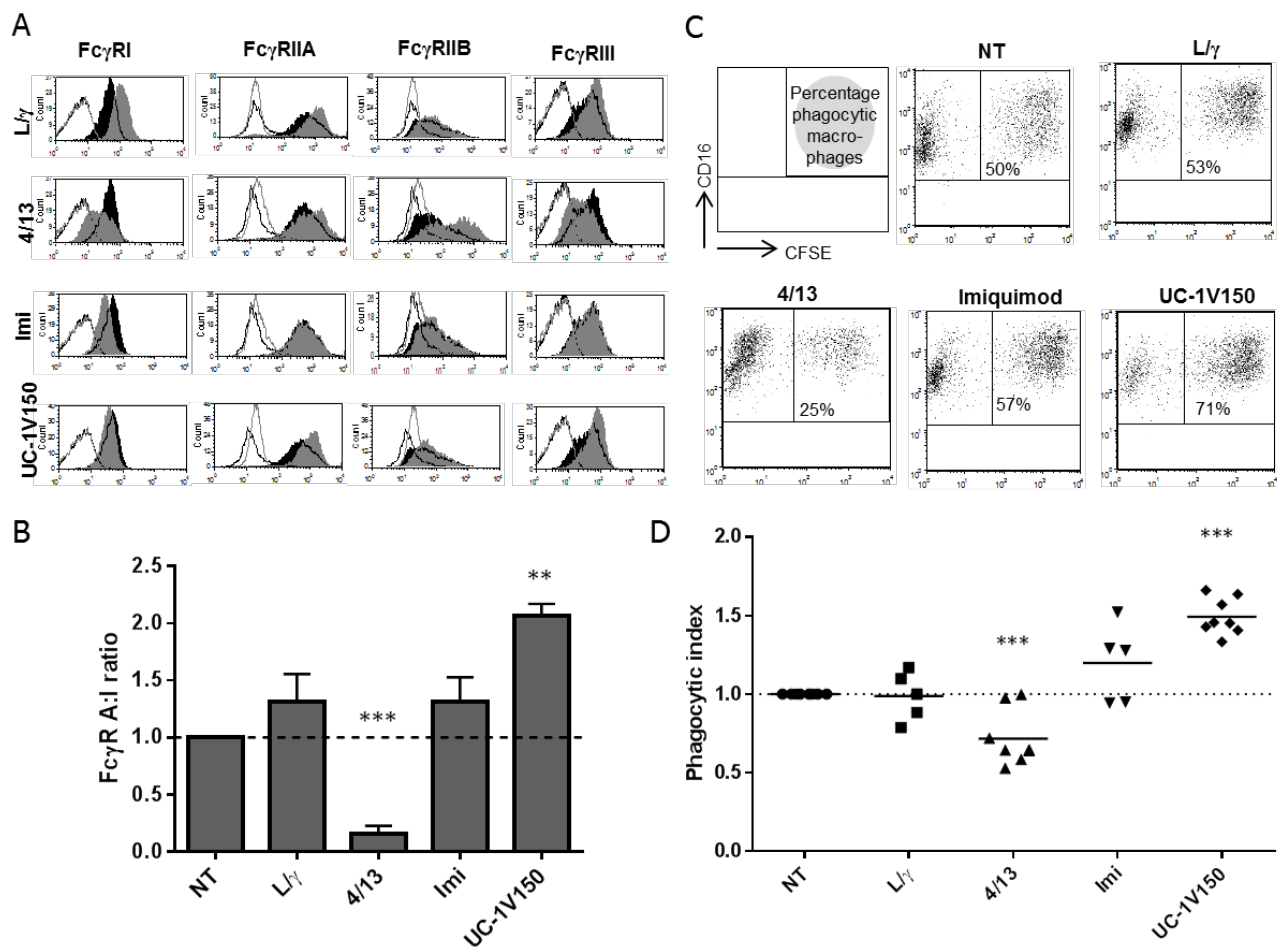


Figure 2

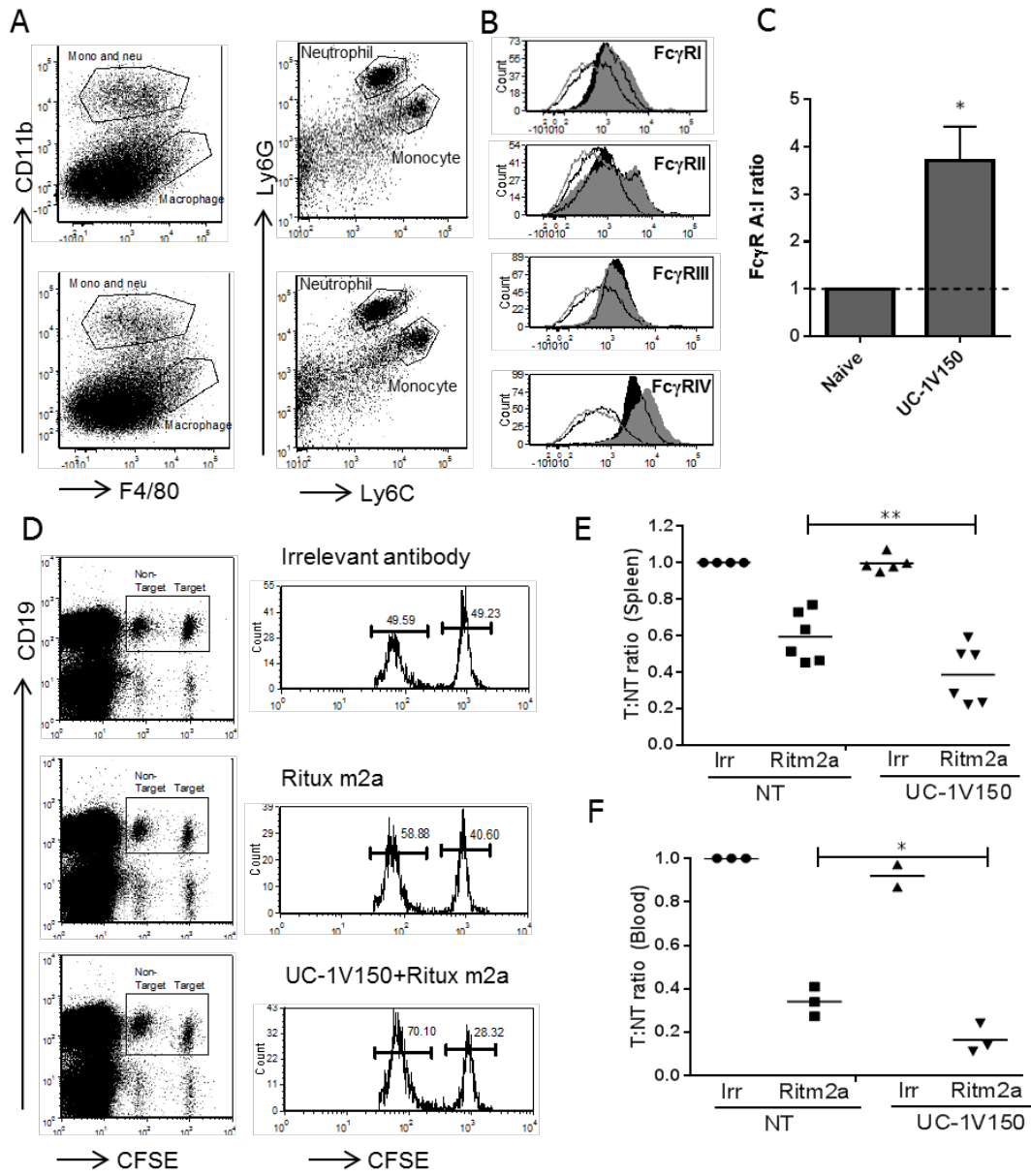


Figure 3