Development and Functional Characterization of NC762, a Novel Therapeutic Antibody Targeting B7-H4, for the Treatment of Malignancies

Abstract #3193

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Background

B7-H4 (B7 homolog 4) is a transmembrane protein in the B7 family of molecules that is expressed on tumor cells. High protein expression on tumors and low expression on healthy tissue makes B7-H4 an attractive molecule for direct targeting. NC762 is a humanized $IgG_{1\kappa}$ monoclonal antibody specific for human B7-H4 that is being developed for the treatment of cancer and demonstrates an excellent safety profile in IND-enabling studies. The Fc region of NC762 contains three point-mutations (S239D/A330L/I332E; DLE) which enhance binding to CD16a in order to increase antibody-dependent cellular cytotoxicity (ADCC). Preclinical data demonstrate that binding of NC762 to tumors expressing B7-H4 results in inhibition of tumor growth *in vivo*. The inhibitory effect on tumor growth is not dependent upon T cells. However, NK cells contribute to enhanced anti-tumor activity mediated by NC762.

NC762 Binds to B7-H4$^+$ Human Cells

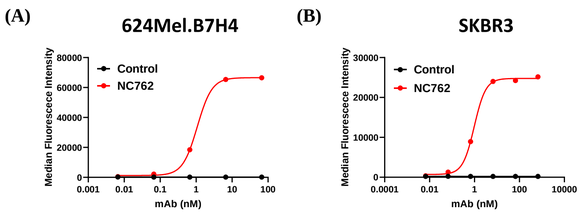


Figure 1: NC762 binds to B7-H4$^+$ human cell lines. Binding of NC762 or an isotype control were measured on (A) 624Mel.hB7H4 cells (EC_{50} = 1.12 nM) or (B) SKBR3 cells (EC_{50} = 0.976 nM) by FACS analysis.

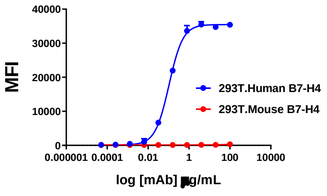


Figure 2: NC762 binds to human but not mouse B7-H4. Binding of NC762 was measured on 293T cells expressing either human B7-H4 or mouse B7-H4 by FACS analysis.

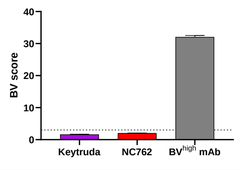


Figure 3: NC762 has low non-specific binding. Binding of NC762, anti-PD-1 antibody Keytruda, or a control antibody with high non-specific binding (BV^{high}) to baculovirus particles was measured.

NC762 Has Increased Binding to CD16a

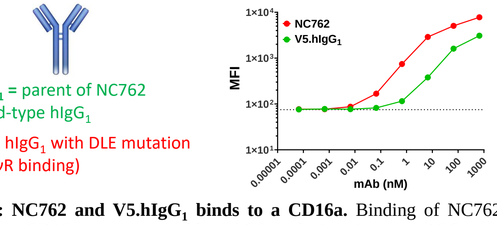


Figure 4: NC762 and V5.hIgG$_1$ binds to a CD16a. Binding of NC762 or V5.hIgG$_1$ to CD16a was measured by FACS analysis using an ADCC reporter cell line that constitutively expresses CD16a.

NC762 Exhibits *In Vitro* ADCC Activity

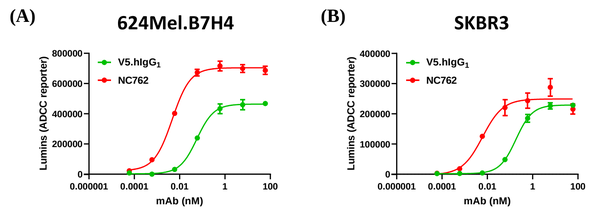


Figure 5: ADCC analysis of NC762 and V5.hIgG$_1$ antibodies using a Jurkat reporter cell line. (A) 624Mel.hB7H4 or (B) SKBR3 target cells were cultured with effector Jurkat reporter cells (2:1 effector to target ratio) with NC762 or V5.hIgG$_1$ at indicated concentrations at 37ºC. Luminescence was measured following five hours of incubation.

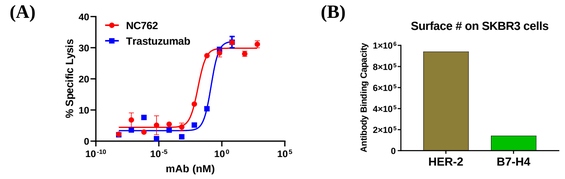
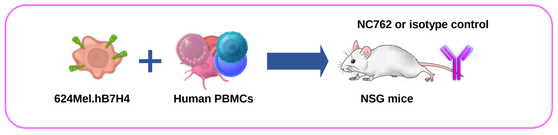


Figure 6: ADCC analysis of NC762 using primary effector cells. (A) Human effector PBMCs were incubated with labeled target SKBR3 cells (20:1 effector to target ratio) in the presence of NC762 or trastuzumab at indicated concentrations at 37ºC for two hours. (B) Antibody binding capacity of HER-2 and B7-H4 were measured on SKBR3 cells.

NC762 Exhibits Dose-dependent *In Vivo* Activity



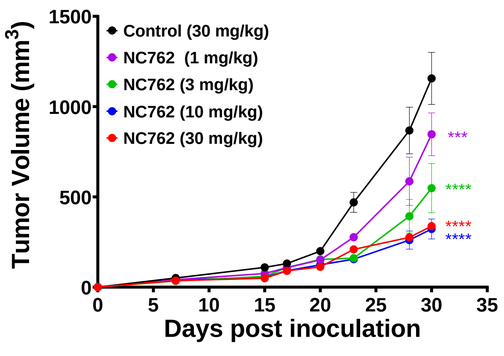


Figure 7: NC762 exhibits dose-dependent activity in a mouse tumor model. Human PBMCs were cultured in the presence of IL-7 and IL-2 for three days. 1E06 624Mel.hB7H4 cells were mixed with 3E05 human PBMCs (3:1 ratio), and inoculated SC into NSG mice on day 0. Mice were treated IP with NC762 at 1 mg/kg, 3 mg/kg, 10 mg/kg, 30 mg/kg, or isotype control hIgG$_1$.DLE at 30 mg/kg, Q7D, 4 doses, starting on day 3 post tumor inoculation. Two-way ANOVA was used to measure significance on day 30. ***P < 0.001, ****P < 0.001

NK Cells Contribute to NC762 Activity

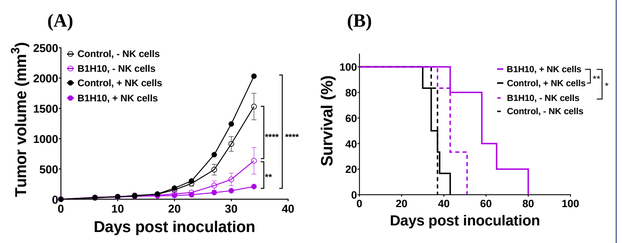


Figure 8: NK cells are involved in anti-B7-H4 mediated tumor growth reduction and prolonged animal survival in a xenograft model. Human PBMCs were cultured in the presence of IL-7 and IL-2 for three days. NK cells were depleted using a MACS column. 1E06 624Mel.hB7H4 cells were mixed with either 1E06 total or NK-cell depleted PBMCs (1:1 ratio) and SC inoculated into NSG mice. Mice were treated IP with parental NC762 clone, B1H10.hIgG$_1$ or isotype control hIgG$_1$ at 10 mg/kg of anti-B7-H4 mAb starting on day 1, Q4D, 7 doses. Two-way ANOVA was used to measure significance. Log-rank Mantel-Cox test was used to analyze survival curves. *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.001

NC762 Restricts Tumor Growth without PBMCs

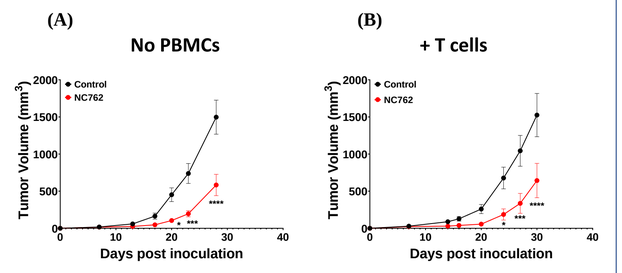


Figure 9: NC762 has activity in the absence of PBMCs. 1E06 624Mel.hB7H4 cells were inoculated SC in (A) the absence of PBMCs or (B) the presence of human T cells (2:1 tumor to T cell ratio) into NSG mice on day 0. Mice were treated IP with NC762, isotype control hIgG$_1$.DLE at 10 mg/kg, Q7D, 4 doses, starting on day 3 post tumor inoculation. Two-way ANOVA was used to measure significance. *P < 0.05, ***P < 0.001, ****P < 0.001

FcγR Binding is Not Required for NC762 Activity

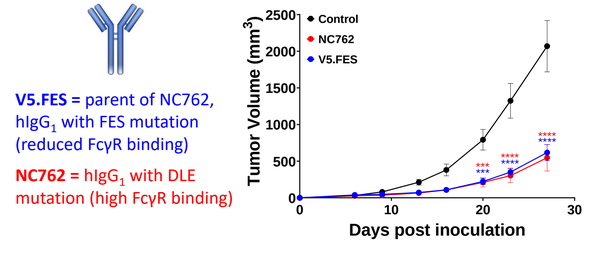
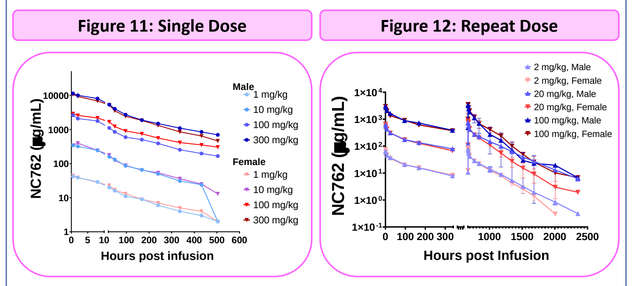


Figure 10: Low affinity Fc does not impact NC762 activity. NSG mice were inoculated SC with 1E06 624Mel.hB7H4 cells on day 0. Treatment with 10 mg/kg of NC762, V5.FES, or PBS control on day 3, Q7D, 4 doses. Two-way ANOVA was used to measure significance. ***P < 0.001, ****P < 0.001

IND Enabling Studies for NC762



Single dose, and dose-range finding study in NHPs

- **Figure 11**: Single dose: (1 Male/1 Female), 30 min IV infusion
  - $T_{1/2}$ is ~ 8.2 days
  - HNSTD* is 300 mg/kg
- **Figure 12**: Repeat dose: (5 Male/5 Female). 3 doses Q2W, 10 W recovery
  - $T_{1/2}$ is ~ 8-10.5 days
  - HNSTD* is 100 mg/kg
  - Histopathology shows no major findings
  - No significant change in serum cytokines
  - No safety concerns

Tissue cross reactivity study on 37 tissues from 3 donors

- No safety concerns

Human *in vitro* cytokine release assays

- No change in cytokines

*HNSTD = highest non-severely toxic dose

B7-H4 expression in tumors

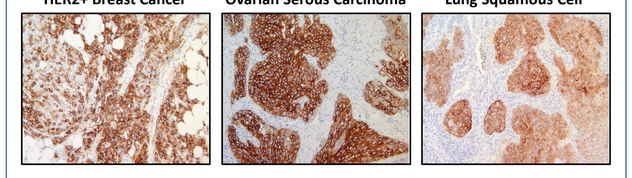


Figure 13: B7-H4 is expressed in tumors. Positive B7-H4 immunohistochemistry (IHC) staining using anti-B7-H4 antibody clone EPR20236 (AbCam) in HER2+ breast cancer (left), ovarian serous carcinoma (middle), and lung squamous cell (right) tumor slides. The greatest prevalence of high B7-H4 expression cases were observed in these three tumor indications. Data provided by Discovery Life Sciences (Newtown, PA).

Conclusion

- NC762 demonstrates great specificity to human B7-H4
- NC762 restricts tumor growth in a xenograft murine tumor model in a dose-dependent manner
- NC762 binds to B7-H4$^+$ cells and promotes ADCC activity *in vitro*
- Depleting NK cells partially reduces activity, however NC762 significantly reduces tumor growth in the absence of human PBMCs
- T cells are not required for *in vivo* NC762 activity
- A mutant of NC762 with low FcγR binding affinity showed similar activity as NC762 with high FcγR binding affinity, suggesting an ADCC-independent mechanism of tumor growth restriction
- Half-life in monkeys is approximately 8 - 10 days
- No safety concerns from monkey toxicology studies or human cytokine release assays