Abstract #TPS3166: A Phase 1/2, Open-Label, Dose-Escalation, Safety and Tolerability Study of NC318 in Subjects with Advanced or Metastatic Solid Tumors

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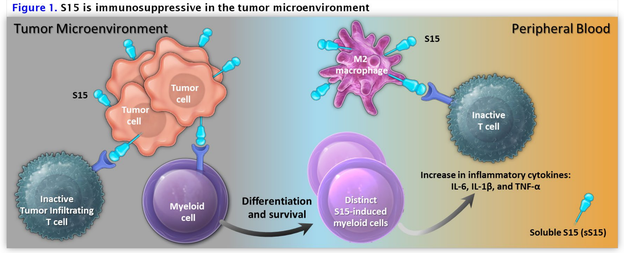
BOX 1: Background

- Siglec-15 (S15) is a member of the Siglec family (Sialic acid-binding Immunoglobulin Lectins), a distinct subgroup of immunoglobulin (Ig) superfamily proteins involved in immune regulation (Macauley MS et al. 2014).
- Nonclinical models demonstrate S15 mediates suppression of T cell proliferation and negatively regulates T cell function. (**Figure 1**)
- NC318 is a first-in-class monoclonal antibody that blocks S15-mediated immune suppression and prevents tumor growth by normalizing T cell function and restoring anti-tumor immunity in the tumor microenvironment (Wang J et al. 2019).
- NC318-01 is a first in human (FIH) multi-center Phase 1/2 study investigating NC318 in patients with advanced/metastatic solid tumors refractory or resistant to treatment (**Figure 2**)
- Preliminary Phase 1 data for this clinical trial were previously presented at the 2019 SITC Annual Meeting (Tolcher A et al. 2019); Phase 2 enrollment is ongoing

Figure 1. S15 is immunosuppressive in the tumor microenvironment



BOX 3: Tumor biopsy and TME characteristics at baseline

Table 3a. S15 expression from screening biopsies

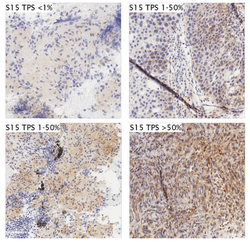
Screening Biopsies Analyzed	S15+ (TC) S15+ (IC)	S15+ (TC) S15-neg (IC)	S15-neg (TC) S15+ (IC)	S15-neg (TC) S15-neg (IC)
15	4	2	2	7

Table 3b. S15 and PD-L1 Expression from Screening Biopsies*

Screening Biopsies Analyzed	S15+ PD-L1+	S15+ PD-L1-neg	S15-neg PD-L1+	S15-neg PD-L1-neg
15	6	2	4	3

TC = Tumor Cells; IC = Immune Cells
[a]Positivity defined as >1% of tumor or immune cell staining at any intensity

Figure 3. Representative S15 staining at screening



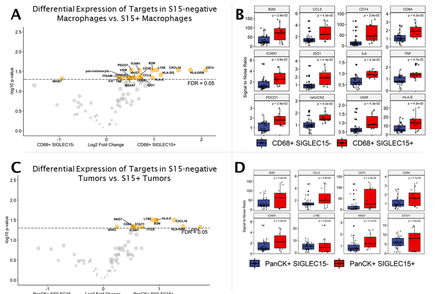


Figure 4. S15 expression on tumors and tumor associated macrophages is associated with other markers of tumor progression and immune suppression. (This analysis is ongoing with enrollment)
5-20 Regions of interest (ROI) each from 6 Phase 1 tissue samples at screening were selected based on varied expression levels determined by immunofluorescence staining of markers of interest (panCK, CD45,CD68 and DAPI). mRNA expression was analyzed (NanoString) with pan-cancer IO panel along with S15 added to the panel. Volcano plot analyzing fold change in individual genes (orange: p<.05) along with their signal to noise ratio in (A-B) CD68+S15+ versus CD68+S15- and (C-D) panCK+S15+ versus PanCK+S15- regions are shown.

All analyses are ongoing

BOX 4: Tumor Biopsy and TME characteristics over time

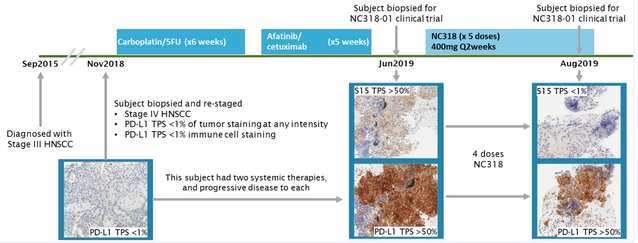
Table 4. S15 and PD-L1 expression from Phase 1 screening and on-treatment biopsies

Diagnosis NC318 Dose	Region of Biopsy	Siglec-15 Screen	Siglec-15 Cycle 3	PD-L1 Screen	PD-L1 Cycle 3
NSCLC (80 mg)	Tumor Cells	-	-	-	-
	Immune Stroma	-	-	+	+
NSCLC (80 mg)	Tumor Cells	-	+	-	+
	Immune Stroma	-	+	-	+
Ovarian (80 mg)	Tumor Cells	-	-	+	-
	Immune Stroma	-	-	-	-
Melanoma (80 mg)	Tumor Cells	+	-	-	-
	Immune Stroma	+	+	+	-
NSCLC (80 mg)	Tumor Cells	-	-	+	+
	Immune Stroma	-	-	+	+
NSCLC (240 mg)	Tumor Cells	-	-	-	-
	Immune Stroma	-	+	-	-
NSCLC (240 mg)	Tumor Cells	+	+	+	-
	Immune Stroma	+	+	-	-
HNSCC (400 mg)	Tumor Cells	+	-	+	+
	Immune Stroma	-	-	+	-
HCC (400 mg)	Tumor Cells	+	-	+	-
	Immune Stroma	-	-	-	+

Positivity in >1% of Tumor Cells or Immune Cells

All analyses are ongoing

Figure 5. S15 and PD-L1 expression may be dynamic over time (representative subject from Phase 1)



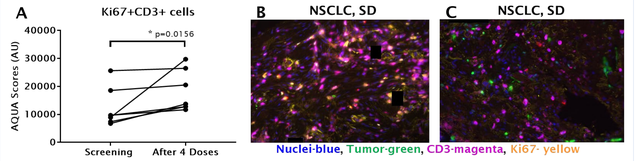
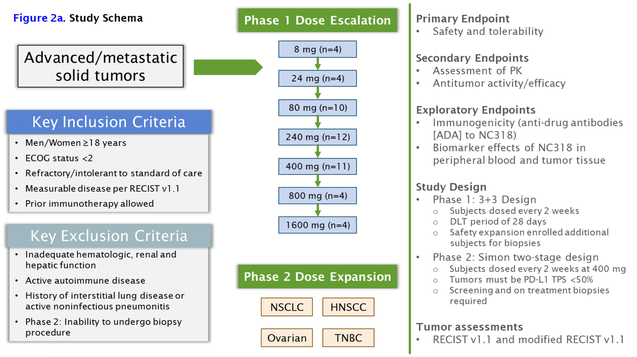


Figure 6. NC318 treatment may be associated with proliferation of T cells in the tumor biopsy, this analysis is ongoing with enrollment. Quantification of CD3+Ki67+ cells from multiplex immuno-fluorescence (MIF) imaging of the tumor microenvironment (TME) of (A) seven available paired biopsies at baseline and after four doses of NC318 (post-treatment). Statistics were analyzed by Wilcoxon test. Example of MIF of the TME of two NSCLC patients with SD showing (B) high or (C) low CD3+Ki67+ staining.

BOX 2: Study Schema and Phase 1 Subjects' Demographics (Phase 2 still enrolling)

Clinical Trial Registry Number: NCT03665285

Figure 2a. Study Schema



Key Inclusion Criteria
- Men/Women ≥18 years
- ECOG status <2
- Refractory/intolerant to standard of care
- Measurable disease per RECIST v1.1
- Prior immunotherapy allowed

Key Exclusion Criteria
- Inadequate hematologic, renal and hepatic function
- Active autoimmune disease
- History of interstitial lung disease or active noninfectious pneumonitis
- Phase 2: Inability to undergo biopsy procedure

Primary Endpoint
- Safety and tolerability

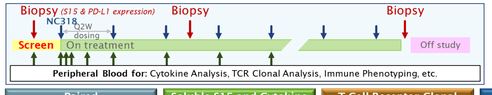
Secondary Endpoints
- Assessment of PK
- Antitumor activity/efficacy

Exploratory Endpoints
- Immunogenicity (anti-drug antibodies [ADA] to NC318)
- Biomarker effects of NC318 in peripheral blood and tumor tissue

Study Design
- Phase 1: 3+3 Design
  - Subjects dosed every 2 weeks
  - DLT period of 28 days
  - Safety expansion enrolled additional subjects for biopsies
- Phase 2: Simon two-stage design
  - Subjects dosed every 2 weeks at 400 mg
  - Tumors must be PD-L1 TPS <50%
  - Screening and on treatment biopsies required

Tumor assessments
- RECIST v1.1 and modified RECIST v1.1

Figure 2b. Biomarker schema: peripheral blood and tumor biopsies collected at screening and while on treatment. ***Designed to assess any changes in biomarkers associated with the S15 immunosuppressive pathway***



15 Phase 1 subjects consented to biopsy collection. Biopsies are required for the ongoing Phase 2 component.

Paired Biopsies	Soluble S15 and Cytokine Analysis	T-Cell Receptor Clonal Analysis	Immune Phenotyping
Assess changes to TME after four doses of NC318 (Figures 3-6)	Relationship of sS15 and cytokines (Figure 7)	Detect expansion of TCR clones over first 3 doses (Figure 9)	Assess changes in immune cells over 16 doses (Figures 8 and 10)

TME: Tumor microenvironment

Table 1. Baseline Demographics: Phase 1 Subjects*

Patient characteristics	Phase 1 subjects (N=49)	Phase 1 NSCLC (n=13)
Age, years		
Median (range)	62 (32-78)	68 (48-77)
Gender, n (%)		
Female	28 (57)	6 (46)
Male	21 (43)	7 (54)
ECOG performance status, n (%)		
0	16 (33)	2 (15)
1	33 (67)	11 (85)
Prior systemic anti-cancer regimens		
Median (range)	3 (1-15)	4 (1-7)
Prior Immunotherapy, n (%)	31 (63)	13 (100)

[a]All comers regardless of PD-L1 or S15 status

Table 2. Enrolling tumor types: Phase 1 subjects*

Phase 1 Tumor Types	n	Biopsied
Non small cell lung (NSCLC)	13	5
Melanoma	7	3
Ovarian cancer	7	2
Breast cancer	4	0
Colorectal cancer	3	1
Other:		
Endometrial	2	0
Gastric	2	0
Head & neck cancer (HNSCC)	2	2
Leiomyosarcoma	2	0
Pancreatic	2	1
Esophageal	1	0
Hepatocellular carcinoma	1	1
Merkel cell carcinoma	1	0
Small cell lung cancer	1	0
Squamous cell carcinoma	1	0

[a]All comers regardless of PD-L1 or S15 status

BOX 5: Soluble S15, Cytokines, NLR, and TCR Clonal Analysis

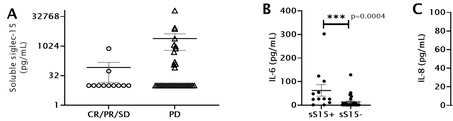


Figure 7. Ongoing analysis: baseline detection of soluble S15 in peripheral blood may be associated with pro-inflammatory cytokines. Baseline soluble S15 detected in 14 of 47 subjects illustrated based on outcome (A). Soluble S15 is associated with higher IL-6 (B) and IL-8 (C) at baseline among 42 subjects analyzed to date. Statistics were analyzed by Mann-Whitney test. (sS15 = Soluble S15, CR = Complete Response, PR = Partial Response, SD = Stable Disease >24 weeks, PD = Progressive Disease)

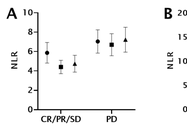


Figure 8. Neutrophil to lymphocyte ratio (NLR) as observed over treatment. Baseline and longitudinal plot of NLR over time for all phase 1 subjects (A; n=49) and NSCLC subjects (B; n=13). This analysis is ongoing.

●= baseline, ■= 14 days, ▲= 28 days.

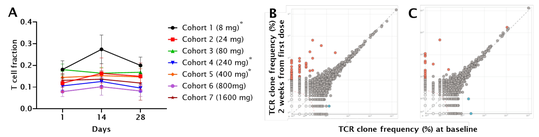


Figure 9. T-Cell receptor (TCR) clonal analysis of phase 1 subjects (n = 49) suggests NC318 mediated immune activation. This analysis is ongoing and will include subjects currently enrolling. Relative change in (A) T cell fraction from baseline compared to T cell fraction at 14 or 28 days after first dose. *Statistically significant difference was seen at 8 mg dosage group between day 1 to 14 (p = 0.0076) and day 14 to 28 (p = 0.02); cohort 4 (240 mg) between day 1 to 14 (p = 0.006) and day 14 to 28 (p = 0.002); and cohort 5 (400 mg) between day 14 to 28 (p = 0.012). Linear mixed model was used for statistical analysis. Examples of TCR clonal expansion in peripheral blood are shown (B-C) for 2 different NSCLC subjects. (Orange circles represent new clones or statistically significant expansion of TCR clones while on treatment, blue circles represent statistically significant decrease of TCR clones while on treatment)

BOX 6: Immune Phenotyping

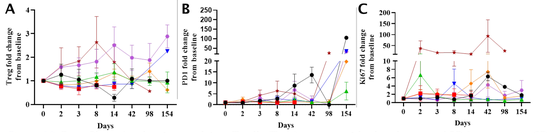


Figure 10. From Phase 1 subjects, regulatory T cells (Treg) increase at higher doses, and PD-1 expressing CD4+ T cells increase over time. Changes in (A) Treg, (B) PD-1 expression within CD4+ T-cell subset; (C) Ki67 expression within CD8+ T cell subset examined over time after NC318 treatment (n=49). Whole blood samples were analyzed using a multicolor flow cytometry. Cells gated on CD3+CD4+ subset was analyzed for (A) CD25+CD127- subset, (B) PD-1; and CD3+CD8+ subset was analyzed for (C) Ki67. (this analysis is ongoing)

Summary of Ongoing Trial and Biomarker Analyses

Phase 1 Clinical Summary of this ongoing trial
- NC318 has been well tolerated across multiple dose levels and no new safety signals have emerged in the Phase 2 component of this trial; immune related adverse events (irAEs) continue to be observed at a frequency consistent with what was previously reported
- As of 11May2020, two NSCLC subjects remain on study: a CR and a PR for 82 weeks and 54 weeks, respectively
- 10 subjects had durable stable disease for at least 24 weeks but have progressed

Biomarker Summary
- Ongoing pharmacodynamic biomarker assessments suggest NC318 activity is demonstrated collectively by immune phenotyping, TCR clonal analysis, and increase of CD3+Ki67+ in TME
- S15 expression can be assessed from tumor biopsies and is still under development
- S15 expression may be an important patient selection criteria for NC318

Future Directions
- Identification of tumors that express S15 (e.g. mCRPC and advanced/metastatic urothelial cancer) may be further evaluated in future clinical trials
- Phase 2 is actively enrolling, and topline data will be presented at a future meeting

Clinical Trial Registry number: NCT03665285

BOX 7: Future Directions

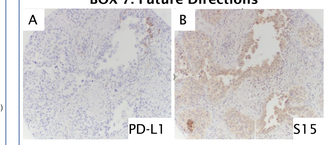


Figure 11. S15 expression in urothelial supports rationale to evaluate NC318 in these patients. PD-L1 low (A) and S15 high (B) expression was observed in urothelial cancer specimens from curative intent cystectomies. S15 expression was associated with a lower CD8 density (p=0.045), with 10/16 cases having a CD8 score of 0.

Rabbit polyclonal antibody supplied by Abcam (ab174732)

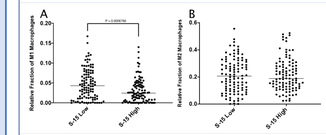


Figure 12. S15 may generate an immunosuppressive TME by suppressing M1 macrophage polarization in mCRPC. S15 mRNA correlates with immunologic signatures of reduced M1 macrophages (A) in the setting of intact M2 macrophages (B). These findings may support evaluation of NC318 for patients with mCRPC.

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Citations:
Macauley M S, et al. (2014). Siglec-mediated regulation of immune cell function in disease. Nat Rev Immunol, 14(10)
Tolcher A, et al. (2019). Single agent anti-tumor activity in PD-1 refractory NSCLC: phase 1 data from the first-in-human trial of NC318, a Siglec-15-targeted antibody. SITC Annual Meeting, 2019
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