732 A NOVEL NUCLEAR RECEPTOR 4A1 (NR4A1) ANTAGONISTS ATTENUATES T-CELL EXHAUSTION IN COLORECTAL CANCER

[1]Kumaravel Mohankumar*, [1]Gus Wright, [1]Subhashree Kumaravel, [1]Rupesh Shrestha, [2]Maen Abdelrahim, [1]Robert Chapkin, [1]Stephen Safe. *[1]Texas A&M University, College Station, TX, United States; [2]Houston Methodist Cancer Center, Houston, TX, United States*

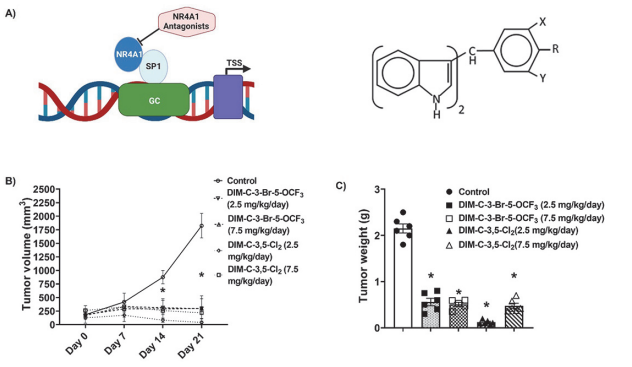
Background Colorectal cancer (CRC) is a highly complex disease with multiple risk factors and both genetic and environmental components contribute to disease incidence.[1 2] Cancer immunotherapy using immune-checkpoint blockades represents a major advance in treatment strategy.[3 4] The orphan nuclear receptor 4A1 (NR4A1) is overexpressed in lung, colon, liver and breast cancers and in Rhabdomyosarcoma and is a negative prognostic factor for cancer patient survival.[5–8] Previous studies in breast cancer cells showed that PD-L1 was regulated by NR4A1 which activates transcription factor Sp1 bound to the PD-L1 gene promoter. Genome-wide studies have identified NR4A1 as a key mediator of T-cell dysfunction and NR4A1 also plays an important role in regulating genes which are involved in tumor-induced T-cell exhaustion.[9] Bis-indole derived NR4A1 ligand that act as receptor antagonists have been developed in this laboratory and these compounds block pro-oncogenic NR4A1-regulated genes/pathways.

Methods Immune competent C57BL/6 mice and mouse MC-38 colon cancer cells were used and tumor Infiltrating Lymphocytes (TILs) were isolated from mice either untreated or treated with CDIM/NR4A1 antagonists. FACS analysis and Real -Time PCR were performed to determine expression of exhaustion markers in these tumor T-cell population.

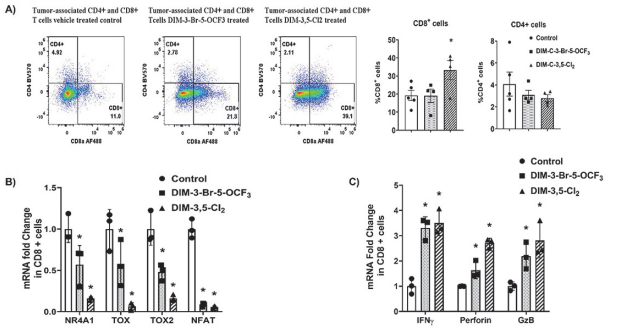
Results

Two compounds: 1,1-bis(3□-indolyl)-1-(3-bromo-5-trifluoromethoxyphenyl)methane (DIM-3-Br-5-OCF3) and 3,5-dichlorophenyl analog (DIM-3,5-Cl2) inhibited tumor growth and weight at doses of 2.5 and 7.5mg/kg/day (figure 1). Tumor CD8+ T-cells isolated from mice treated with DIM-3,5-Cl2 and DIM-3-Br-5-OCF3 exhibited decreased mRNA expression of NR4A1 and high mobility group – box transcription factors NFAT, TOX and TOX2 and increased mRNA levels of Interferon-γ (IFNγ), granzyme β (GzB) and perforin compared to control animals (figure 2). As TOX and TOX2 cooperate with NR4A1 to modulate CD8+ T-cell exhaustion, we investigated the expression of several inhibitory receptors of T-cell exhaustion in CD8+ TILs, including PD-1, 2B4, TIGIT and TIM3. Following treatment with DIM-3,5-Cl2 or DIM-3-Br-5-OCF3, there was a significant decrease in the percentage of PD1 and 2B4 cells and a decrease in TIGIT and TIM3 (figure 3). These results indicate that NR4A1 antagonists reverses T-cell exhaustion.

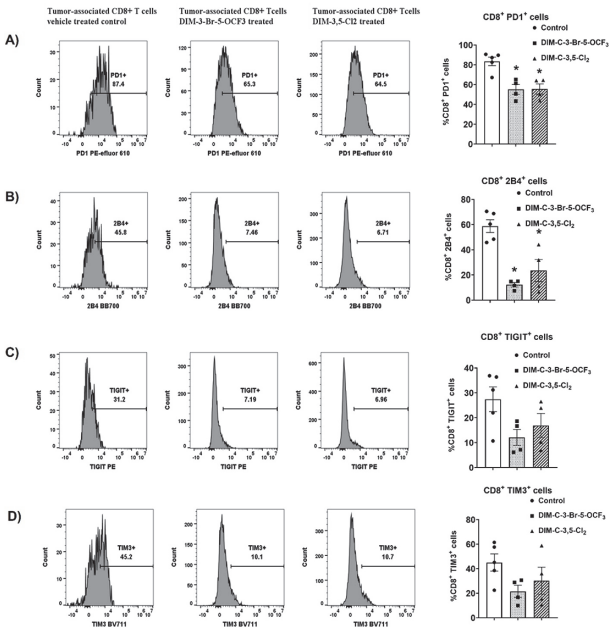
Conclusions NR4A1 plays a critical role in T-cell dysfunction, and this includes T-cell exhaustion.[10 11] Our results demonstrate that the NR4A1 antagonists reverse many markers of T-cell exhaustion including activation of cytokines. The combined effects of NR4A1 antagonists in both tumors and T-cells result in inhibition of colon tumorigenesis by targeting pathways/genes in tumor cells and by enhancing immune surveillance via reversal of T-cell exhaustion.



Abstract 732 Figure 1 CDIM/NR4A1 antagonists inhibit colon tumor growth. Model for regulation of genes with GC-rich promoters by NR4A1/SP1 (A). C57BL/6 mice bearing MC-38 cells as xenografts were treated for 21 days with corn oil (control), DIM-3-Br-5-OCF3 (2.5 and 7.5 mg/kg/d), DIM-3,5-Cl2 (2.5 and 7.5 mg/kg/d) and effects on tumor volume (B), and tumor weights (C) were determined.



Abstract 732 Figure 2 CDIM analogs alter transcription factors expression. FACS analysis and CD4 and CD8 – specific antibodies were used to determine T-cell population in tumors of mice treated with corn oil (control), DIM-3-Br-5-OCF3 (2.5 and 7.5 mg/kg/d) and DIM-3,5-Cl2 (2.5 and 7.5 mg/kg/d) (A). Real time PCR was used to determine expression of nuclear factors (B) and cytokine (C) mRNA levels in CD8+ T-cells isolated from tumors. Results are expressed as means ± SD replicates from each treatment group and significant ($p<0.05$) induction or inhibition is indicated (*)



Abstract 732 Figure 3 NR4A1 ligands decreases T-cell exhaustion markers. FACS analysis in tumors derived from mice treated with corn oil (control), DIM-3-Br-5-OCF3 (2.5 and 7.5 mg/kg/d) and DIM-3,5-Cl2 (2.5 and 7.5 mg/kg/d) using specific antibodies was carried out to determine percentage of CD8+ T-cells expressing T-cell exhaustion markers - PD1 (A), 2B4 (B), TIGIT (C) and TIM3 (D). Significant ($p<0.05$) induction or inhibition is indicated (*) and results are expressed as means ± SD for at least 4 separate mice per treatment group.

REFERENCES

1. Ahmed M. Colon cancer: a Clinician's perspective in 2019. *Gastroenterology Res* 2020;**13**(1):1–10. Epub 2020/02/26. doi: 10.14740/gr1239.
2. Keum N, Giovannucci E. Global burden of colorectal cancer: emerging trends, risk factors and prevention strategies. *Nat Rev Gastroenterol Hepatol* 2019;**16**(12):713–32. Epub 2019/08/29. doi: 10.1038/s41575-019-0189-8.
3. Pardoll DM. The blockade of immune checkpoints in cancer immunotherapy. *Nat Rev Cancer* 2012;**12**(4):252–64. Epub 2012/03/23. doi: 10.1038/nrc3239.
4. Ribas A, Wolchok JD. Cancer immunotherapy using checkpoint blockade. *Science* 2018;**359**(6382):1350–1355.
5. Yang et al. Distinct epigenetic features of tumor-reactive CD8+ T cells in colorectal cancer patients revealed by genome-wide DNA methylation analysis. *Genome Biol* 2019;**21**(1):2. doi: 10.1186/s13059-019-1921-y.
6. Safe S, Karki K. The paradoxical roles of orphan nuclear receptor 4A (NR4A) in cancer. *Mol Can Res* 2020. Epub 2020/10/28. doi: 10.1158/1541-7786.MCR-20-0707.
7. Lee SO, et al. Diindolylmethane analogs bind NR4A1 and are NR4A1 antagonists in colon cancer cells. *Molecular Endocrinology*. 2014;**28**(10):1729–39. doi: 10.1210/me.2014-1102.
8. Hedrick E, et al. The nuclear orphan receptor NR4A1 regulates β1-integrin expression in pancreatic and colon cancer cells and can be targeted by NR4A1 antagonists. *Mol Carcinog* 2017;**56**(9):2066–2075.
9. Liu X, et al. Genome-wide analysis identifies NR4A1 as a key mediator of T cell dysfunction. *Nature* 2019;**567**(7749):525–9. Epub 2019/03/01. doi: 10.1038/s41586-019-0979-8.
10. Chen J et al. NR4A transcription factors limit CAR T cell function in solid tumours. *Nature*. 2019;**567**(7749):530–4. Epub 2019/03/01. doi: 10.1038/s41586-019-0985-x. PubMed PMID: 30814732; PMCID: PMC6546093.
11. Seo H, et al. TOX and TOX2 transcription factors cooperate with NR4A transcription factors to impose CD8(+) T cell exhaustion. *Proc Natl Acad Sci USA* 2019;**116**(25):12410–5.

Ethics Approval All animal studies were carried out according to the ethical procedures approved by the Texas A&M University Institutional Animal Care and Use Committee. Approval number is 2020-0138.

http://dx.doi.org/10.1136/jitc-2021-SITC2021.732