

**COMBINATORIAL APPROACHES TO MITIGATE THE  
CHALLENGES WITH CD40 AGONIST ANTIBODY FOR  
DURABLE ANTI-TUMOR IMMUNITY**

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**Combinatorial Approaches to Mitigate the Challenges with  
CD40 Agonist Antibody for Durable Anti-Tumor Immunity**

**A Thesis**

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श्री अद्वैत, गदाधर, श्रीवास आदि गौर भक्त वृन्द ॥

*Maa, thank you for being my unwavering support, care and strength throughout my life.*

*Papa, wherever you are, I hope I've made you proud and happy.*

*Aditi, I'm grateful for your motherly care, immense support and love.*

*Sushil, I will forever be grateful for all that you have done for me and our family*

## Certificate

This is to certify that the thesis entitled “**Combinatorial approaches to mitigate the challenges with CD40 agonist antibody for durable anti-tumor immunity**” has been carried out by Mr. Vidit Gaur, a bonafide Ph.D. student at Centre for Bio-medical Engineering, Indian Institute of Technology Delhi. The conceptualization, investigations, observations, and conclusions reported in the thesis are based on his original work conducted under my direct supervision. This thesis, being submitted for the award of Ph.D. degree has not been submitted in part or in full to this or any other University for the award of any degree or diploma.



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## Declaration

The thesis entitled “**Combinatorial approaches to mitigate the challenges with CD40 agonist antibody for durable anti-tumor immunity**” has been carried out under the supervision of Dr. Jayanta Bhattacharyya and submitted for the fulfilment of Ph.D. in the Centre for Bio-medical Engineering, Indian Institute of Technology Delhi. The research comprises of original work that has not been submitted previously in part or full to this or any University for award of academic degree or diploma.

I hereby solemnly declare that all information provided in this thesis are obtained and presented in compliance with the academic rules of ethical conduct.



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## Abstract

CD40, a member of the tumor necrosis factor receptor superfamily, plays a critical role in mediating immune cell communication and serves as a bridge between innate and adaptive immunity. It is widely expressed on the surface of DCs, macrophages, B cells and a wide range of tumors. Upon binding with its trimeric ligand (CD40L) which is expressed on activated T cells, CD40 promotes the activation and differentiation of APCs, resulting in the generating robust anti-tumor responses. In particular, CD40 stimulation on DCs can induce the expression of MHC molecules and co-stimulatory molecules (CD80, CD86, etc) and can re-educate macrophages to induce apoptosis of cancer cells. Hence, the activation of CD40 through its ligation is well-known to impart anti-tumor effects, either directly or indirectly, by stimulating both adaptive and innate immune cells. Therefore, CD40 emerges as a promising therapeutic target for cancer immunotherapy.

Over the past two decades, CD40 agonists have shown promising anti-tumor responses in both pre-clinical and early clinical trials. Among various CD40 agonists, agonist CD40 antibodies ( $\alpha$ CD40) have gained significant attention and shown immense potential to stimulate long-lasting anti-tumor response.  $\alpha$ CD40 mimics the function of CD40L and promotes anti-tumor effects by activating both innate and adaptive immune cells and has shown promising results in both preclinical and clinical studies. However, the use of  $\alpha$ CD40 is associated with certain limitations, which serve as one of the bottlenecks for further clinical development. Various pre-clinical and clinical studies of  $\alpha$ CD40 revealed that the administration of  $\alpha$ CD40 is linked with potential disadvantages like cytokine release syndromes, organ-specific toxicities, autoimmune reactions and thromboembolic syndromes. In addition,  $\alpha$ CD40 administration has been shown to surge the expression of PD-L1, and CTLA-4 in TME leading to the dysfunction and apoptosis of tumor killing immune cells; ultimately causing immune resistance and supporting tumor progression. Moreover, within TME, the low expression of these co-stimulatory molecules (CD40, CD86, and CD80) and the reduced availability of tumor antigens, often caused by antigen masking, significantly hinder the effectiveness of cancer immunotherapy. Therefore, these disadvantages limit the clinical efficacy of  $\alpha$ CD40 and suggest an utmost need to develop some strategies to improve the efficacy while averting its associated toxicities. To this end, in this thesis,

novel therapeutic combinations and formulations were developed to address these issues:

**1. Combination study with cell-free vaccine using Dendritic cell-derived extra-cellular vesicles:** A cell-free vaccine using tumor specific DC-derived extracellular vesicles (DeX) was designed and combined with  $\alpha$ CD40 to enhance the anti-tumor efficacy. The combination therapy significantly delayed the tumor growth and showed 40% tumor-free survival.  $\alpha$ CD40/DeX combination showed modulation of tumor microenvironment and significantly reduced lung metastasis.

**2. Combinatorial approach with tyrosine kinase inhibitor:** Ponatinib, a tyrosine kinase inhibitor, boosted the anti-tumor efficacy of  $\alpha$ CD40 while limiting the associated toxicities. The combination therapy improved the overall survival across different tumor models, reduced PD-L1 expression and averted  $\alpha$ CD40 associated toxicities.

**3. CD40 agonist antibody engineered immunosomes:** A novel formulation (Immunosomes) was engineered to enable selective delivery of  $\alpha$ CD40 and RRX-001 to tumors. Immunosomes significantly modulated TME, surged pro-inflammatory cytokines, showed 100% tumor-free survival and reduced associated toxicities. Most interestingly, Immunosomes generated long-lasting immune memory that prevented future tumour recurrence without further treatment.

## सार

CD40, ट्यूमर नेक्रोसिस फैक्टर रिसेप्टर सुपरफैमिली का एक सदस्य है, जो प्रतिरक्षा कोशिका संचार में मध्यस्थता करने में महत्वपूर्ण भूमिका निभाता है और जन्मजात और अनुकूली प्रतिरक्षा के बीच एक पुल के रूप में कार्य करता है। यह डीसी, मैक्रोफेज, बी कोशिकाओं और ट्यूमर की एक विस्तृत श्रृंखला की सतह पर व्यापक रूप से व्यक्त किया जाता है। सक्रिय टी कोशिकाओं पर व्यक्त अपने ट्रिमेरिक लिगैंड (CD40L) के साथ बंधने पर, CD40 APCs की सक्रियता और भेदभाव को बढ़ावा देता है, जिसके परिणामस्वरूप मजबूत एंटी-ट्यूमर प्रतिक्रियाएँ उत्पन्न होती हैं। विशेष रूप से, DC पर CD40 उत्तेजना MHC अणुओं और सह-उत्तेजक अणुओं (CD80, CD86, आदि) की अभिव्यक्ति को प्रेरित कर सकती है और कैंसर कोशिकाओं के एपिटोपिस को प्रेरित करने के लिए मैक्रोफेज को फिर से शिक्षित कर सकती है। इसलिए, इसके बंधन के माध्यम से CD40 की सक्रियता, अनुकूली और जन्मजात प्रतिरक्षा कोशिकाओं दोनों को उत्तेजित करके, सीधे या अप्रत्यक्ष रूप से एंटी-ट्यूमर प्रभाव प्रदान करने के लिए जानी जाती है। इसलिए, CD40 कैंसर इम्यूनोथेरेपी के लिए एक आशाजनक चिकित्सीय लक्ष्य के रूप में उभरता है। पिछले दो दशकों में, CD40 एगोनिस्ट ने प्री-क्लिनिकल और शुरुआती क्लिनिकल ट्रायल दोनों में आशाजनक एंटी-ट्यूमर प्रतिक्रियाएँ दिखाई हैं। विभिन्न CD40 एगोनिस्ट में, एगोनिस्ट CD40 एंटीबॉडी ( $\alpha$ CD40) ने महत्वपूर्ण ध्यान आकर्षित किया है और लंबे समय तक चलने वाली एंटी-ट्यूमर प्रतिक्रिया को उत्तेजित करने की अपार क्षमता दिखाई है।  $\alpha$ CD40 CD40L के कार्य की नकल करता है और जन्मजात और अनुकूली प्रतिरक्षा कोशिकाओं दोनों को सक्रिय करके एंटी-ट्यूमर प्रभावों को बढ़ावा देता है और प्रीक्लिनिकल और क्लिनिकल दोनों अध्ययनों में आशाजनक परिणाम दिखाए हैं। हालाँकि,  $\alpha$ CD40 का उपयोग कुछ सीमाओं से जुड़ा हुआ है, जो आगे के नैदानिक विकास के लिए बाधाओं में से एक के रूप में काम करते हैं।  $\alpha$ CD40 के विभिन्न प्री-क्लिनिकल और क्लिनिकल अध्ययनों से पता चला है कि  $\alpha$ CD40 का प्रशासन साइटोकाइन रिलीज़ सिंड्रोम, अंग-विशिष्ट विषाक्तता, ऑटोइम्यून प्रतिक्रियाओं और थ्रोम्बोम्बोलिक सिंड्रोम जैसे संभावित नुकसानों से जुड़ा हुआ है। इसके अलावा,  $\alpha$ CD40 प्रशासन ने TME में PD-L1 और CTLA-4 की अभिव्यक्ति को बढ़ा दिया है, जिससे ट्यूमर को मारने वाली प्रतिरक्षा कोशिकाओं की शिथिलता और अपोप्टोसिस

हो जाती है; अंततः प्रतिरक्षा प्रतिरोध का कारण बनता है और ट्यूमर की प्रगति का समर्थन करता है। इसके अलावा, TME के भीतर, इन सह-उत्तेजक अणुओं (CD40, CD86 और CD80) की कम अभिव्यक्ति और ट्यूमर एंटीजन की कम उपलब्धता, जो अक्सर एंटीजन मास्किंग के कारण होती है, कैंसर इम्यूनोथेरेपी की प्रभावशीलता में काफी बाधा डालती है। इसलिए, ये नुकसान  $\alpha$ CD40 की नैदानिक प्रभावकारिता को सीमित करते हैं और इससे जुड़ी विषाक्तता को रोकते हुए प्रभावकारिता में सुधार करने के लिए कुछ रणनीतियों को विकसित करने की अत्यधिक आवश्यकता का सुझाव देते हैं। इस उद्देश्य से, इस थीसिस में, इन मुद्दों को संबोधित करने के लिए नए चिकित्सीय संयोजन और फॉर्मूलेशन विकसित किए गए:

1. डेंड्रिटिक सेल-व्युत्पन्न एक्स्ट्रा-सेलुलर वेसिकल्स का उपयोग करके सेल-फ्री वैक्सीन के साथ संयोजन अध्ययन: ट्यूमर विशिष्ट डीसी-व्युत्पन्न एक्स्ट्रासेलुलर वेसिकल्स (डीएक्स) का उपयोग करके एक सेल-फ्री वैक्सीन तैयार की गई और एंटी-ट्यूमर प्रभावकारिता को बढ़ाने के लिए  $\alpha$ CD40 के साथ संयुक्त किया गया। संयोजन चिकित्सा ने ट्यूमर के विकास में काफी देरी की और 40% ट्यूमर-मुक्त उत्तरजीविता दिखाई।  $\alpha$ CD40/DeX संयोजन ने ट्यूमर माइक्रोएन्वायरमेंट का मॉड्यूलेशन दिखाया और फेफड़ों के मेटास्टेसिस को काफी कम किया।

2. टायरोसिन किनेज अवरोधक के साथ संयोजन दृष्टिकोण: पोनाटिनिब, एक टायरोसिन किनेज अवरोधक, ने संबंधित विषाक्तता को सीमित करते हुए  $\alpha$ CD40 की एंटी-ट्यूमर प्रभावकारिता को बढ़ाया। संयोजन चिकित्सा ने विभिन्न ट्यूमर मॉडल में समग्र उत्तरजीविता में सुधार किया, PD-L1 अभिव्यक्ति को कम किया और  $\alpha$ CD40 से संबंधित विषाक्तता को टाला।

3. CD40 एगोनिस्ट एंटीबॉडी इंजीनियर्ड इम्यूनोसोम: ट्यूमर में  $\alpha$ CD40 और RRX-001 की चुनिंदा डिलीवरी को सक्षम करने के लिए एक नया फॉर्मूलेशन (इम्यूनोसोम) इंजीनियर किया गया था। इम्यूनोसोम ने TME को महत्वपूर्ण रूप से संशोधित किया, प्रो-इंफ्लेमेटरी साइटोकिन्स को बढ़ाया, 100% ट्यूमर-मुक्त अस्तित्व दिखाया और संबंधित विषाक्तता को कम किया। सबसे दिलचस्प बात यह है कि इम्यूनोसोम ने लंबे समय तक चलने वाली प्रतिरक्षा स्मृति उत्पन्न की जिसने बिना किसी अतिरिक्त उपचार के भविष्य में ट्यूमर की पुनरावृत्ति को रोका।

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Table 1: Different types of CD40 agonists

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## List of Abbreviations

$\alpha$ CD40: Agonistic CD40 monoclonal antibodies

APC: Antigen Presenting Cells

TME: Tumor Microenvironment

DC: Dendritic Cell

DeX: DC-derived Extra Cellular Vesicles

PD-1/PD-L1: Programmed Cell Death Protein-1/Programmed Death-Ligand 1

IFN- $\gamma$ : Interferon Gamma

ALT: Alanine aminotransferase

AST: Aspartate aminotransferase

Tregs: T-regulatory cells

GC: Germinal centres

CTL: Cytotoxic T-lymphocyte

ICI: Immune checkpoint inhibitors

ACT: Adoptive cell therapies

CD40L: CD40 Ligand

CTLA-4: Cytotoxic T-lymphocyte associated protein 4

IFN- $\gamma$ : Interferon-gamma

ICOS: Inducible costimulator

mAbs: Monoclonal antibodies

MHC: Major histocompatibility complex

FOXP3: Forkhead box protein P3

ALP: Alkaline phosphatase

MDSC: Myeloid derived suppressor cells

TAM: Tumor associated macrophages

GM-CSF: Granulocyte monocyte colony stimulating factor