MAXIMIZING SAFETY AND EFFICACY OF BIOTHERAPEUTICS BY CONTROLLING CRITICAL QUALITY ATTRIBUTES

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by

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.....Dedicated to my grandfather

Late Sh. Rajaram Singh

Your ideals and teachings of life will keep guiding me towards the path of Truth and Integrity.

CERTIFICATE

This is to certify that the thesis entitled **"MAXIMIZING SAFETY AND EFFICACY OF BIOTHERAPEUTICS BY CONTROLLING CRITICAL QUALITY ATTRIBUTES"** being submitted by **SUMIT KUMAR SINGH** to the Indian Institute of Technology Delhi for the award of the degree of **Doctor of Philosophy** is a record of the original bonafide research work carried out by him under my guidance and supervision. The results contained in this thesis have not been submitted in part or in full to any other University or Institute for the award of any degree or diploma.

I certify that he has pursued the prescribed course of research.

Prof. Anurag S. Rathore
Department of Chemical Engineering
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ABSTRACT

The number of biologic drugs that are gaining approval across the globe are rapidly increasing. Prominent among these modalities include monoclonal antibodies (mAbs) and growth factors as they constituted 30% and 15% respectively. However, the process to go from the idea to getting marketing approval for a biotech drug is long, expensive and arduous. A major hurdle in the entire exercise is high chances of product failure due to compromised safety and efficacy. Comlicating this further, the number of product attributes that can potentially impact safety and efficacy of a biotech drug are overwhelmingly large. It is a challenge to assess the impact of each attribute on the product's safety and efficacy therefore warranting innovative and novel preclinical approaches are needed to bridge the gap. In this work, product as well as process related impurities associated with mAbs and Granulocyte Colony Stimulating Factor (GCSF) has been investigated with regard to their impact on safety and efficacy. In the first part of the thesis, a risk analysis was performed for various impurities that could be formed as a result of GCSF bioprocessing i.e., oxidized, reduced, aggregates, f-Met forms. Based on the severeity and likelihood of their presence in the drug product, a crtiticality score were assigned to each of these impurities. Oxidation of all the methionine's was recognized as a critical quality attribute (CQA) in GCSF. The mechanism of how methionine oxidation affects the structure and function of GCSF is proposed. Further, a strategy involving substituting methionine with alanine in GCSF using site directed mutagenesis to overcome the loss in efficacy due to oxidation is shown. The second part of thesis investigates whether charge variants in mAbs should be considered as CQAs. An empirical model incorporating charge variant levels and their corresponding biological activity is suggested to obtain product pool with maximum activity and yield. Further, the risks and therapeutic benefits of the most commonly found charge variants in mAbs, i.e, lysine variants were investigated. It was found that though lysine variants of monoclonal antibodies have longer half-lives, yet they exhibit high tendency of tumor metastasis. The last part of the thesis establishes mechanism of co-purification of ten host cell protein (HCP)s impurities in Chinese Hamster Ovary (CHO) cells. The proposed mechanism can be used to guide downstream process design or avenues for protein engineering during product discovery to achieve more effective removal of these persisting HCP impurities.

सार

दुनिया भर में अनुमोदन प्राप्त करने वाली जैविक दवाओं की संख्या तेजी से बढ़ रही है। इन तौर-तरीकों में प्रमुख रूप से मोनोक्लोनल एंटीबॉडी (mAbs) और वृद्धि कारक शामिल हैं क्योंकि वे क्रमशः 30% और 15% का गठन करते हैं। हालांकि, बायोटेक दवा के लिए विपणन की मंजूरी लेने के लिए विचार से विपणन अनुमोदन जाने की प्रक्रिया लंबी, महंगी और कठिन है। संपूर्ण अभ्यास में एक प्रमुख बाधा सुरक्षा और प्रभावकारिता के कारण उत्पाद की विफलता की उच्च संभावना है। इसे और आगे बढ़ाते हुए, एक बायोटेक दवा की सुरक्षा और प्रभावकारिता को संभावित रूप से प्रभावित करने वाले उत्पाद विशेषताओं की संख्या बहुत अधिक हो सकती है। यह उत्पाद की सुरक्षा और प्रभावकारिता पर प्रत्येक गुण के प्रभाव का आकलन करने के लिए एक चुनौती है, इसलिए अंतर को पाटने के लिए नवीन और उपन्यास के पूर्ववर्ती दृष्टिकोणों की आवश्यकता होती है। इस काम में, उत्पाद के साथ-साथ mAbs और ग्रैनुलोसाइट कॉलोनी स्टिमुलेटिंग फैक्टर (GCSF) से जुड़ी प्रक्रिया संबंधी अशुद्धियों को सुरक्षा और प्रभावकारिता पर उनके प्रभाव के संबंध में जांच की गई है। थीसिस के पहले भाग में, विभिन्न अशुद्धियों के लिए एक जोखिम विश्लेषण किया गया था जिसे जीसीएसएफ बायोप्रोसेसिंग के परिणामस्वरूप बनाया जा सकता है, यानी ऑक्सिडीज़ेड, रेडूसेड, अग्रेगेटेड, फ-मेट फॉर्म। दवा उत्पाद में उनकी उपस्थिति की गंभीरता और संभावना के आधार पर, इन अशुद्धियों में से प्रत्येक को एक आलोचनात्मक स्कोर सौंपा गया था। GCSF में सभी मेथिओनिन के ऑक्सीडेशन को एक महत्वपूर्ण गुणवत्ता विशेषता (CQA) के रूप में मान्यता दी गई थी। मेथियोनिन ऑक्सीडेशन की संरचना GCSF की संरचना और कार्य को कैसे प्रभावित करती है, इसका तंत्र प्रस्तावित है। इसके अलावा, जीसीएसएफ में अलनीन के साथ मेथियोनीन को प्रतिस्थापित करने वाली एक रणनीति का उपयोग किया गया है, जिसमें ऑक्सीडेशन के कारण प्रभावकारिता में होने वाले नुकसान को दूर करने के लिए निर्देशित उत्परिवर्तजन का उपयोग किया गया है। थीसिस का दूसरा भाग यह जांच करता है कि mAbs में चार्ज वेरिएंट को CQAs माना जाना चाहिए या नहीं। एक अनुभवजन्य मॉडल जिसमें प्रभारी प्रकार स्तर शामिल हैं और उनकी इसी जैविक गतिविधि को अधिकतम गतिविधि और उपज के साथ उत्पाद पूल प्राप्त करने का सुझाव दिया गया है। इसके अलावा, mAbs में सबसे अधिक पाए जाने वाले चार्ज वेरिएंट के जोखिम और चिकित्सीय लाभों, यानी, लाइसिन वेरिएंट की जांच की गई। यह पाया गया कि हालांकि मोनोक्लोनल एंटीबॉडी के लाइसिन वेरिएंट में ज्यादा हाफ-लाइफ रहता है, फिर भी वे ट्यूमर मेटास्टेसिस की उच्च प्रवृत्ति का प्रदर्शन करते हैं। थीसिस का अंतिम भाग चायीनिज हैम्स्टर ओवरी (CHO) कोशिकाओं में दस होस्ट सेल प्रोटीन (HCP) की अशुद्धियों के सह-शुद्धिकरण का तंत्र स्थापित करता है। प्रस्तावित तंत्र का उपयोग उत्पाद की खोज के दौरान प्रोटीन इंजीनियरिंग के लिए डाउनस्ट्रीम प्रक्रिया डिजाइन का

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