NEOPLASIA
www.neoplasia.com

Volume 19 Number xx Month 2017 pp. 661–671 661

The Antitumor Activity of IMGN529, a CD37-Targeting Antibody-Drug Conjugate, Is Potentiated by Rituximab in Non-Hodgkin Lymphoma Models

Stuart W. Hicks, Katharine C. Lai, L. Cristina Gavrilescu, Yong Yi, Surina Sikka, Prerak Shah, Meghan E. Kelly, Jenny Lee, Leanne Lanieri, Jose F. Ponte, Callum M. Sloss and Angela Romanelli

ImmunoGen, Inc., Waltham, MA

Abstract

Naratuximab emtansine (IMGN529) is an investigational antibody-drug conjugate consisting of a CD37-targeting antibody conjugated to the maytansine-derived microtuble disruptor, DM1. IMGN529 has shown promising preclinical and clinical activity in non-Hodgkin lymphoma, including diffuse large B-cell lymphoma (DLBCL). Due to the aggressive nature of the disease, DLBCL is often treated with combination therapies to maximize clinical outcomes; therefore, we investigated the potential of combining IMGN529 with both standard-of-care and emerging therapies against multiple oncology-relevant targets and pathways. The strongest enhancement in potency was seen with anti-CD20 antibodies, including rituximab. The combination of IMGN529 and rituximab was more potent than either agent alone, and this combinatorial benefit was associated with increased apoptotic induction and cell death. Additional studies revealed that rituximab treatment increased the internalization and degradation of the CD37-targeting antibody moiety of IMGN529. The combination of IMGN529 and rituximab was highly efficacious in multiple xenograft models, with superior antitumor efficacy seen compared to either agent alone or treatment with R-CHOP therapy. These findings suggest a novel mechanism whereby the potency of IMGN529 can be enhanced by CD20 binding, which results in the increased internalization and degradation of IMGN529 leading to the generation of greater amounts of cytotoxic catabolite. Overall, these data provide a biological rationale for the enhanced activity of IMGN529 in combination with rituximab and support the ongoing clinical evaluation of IMGN529 in combination with rituximab in patients with relapsed and/or refractory DLBCL.

Neoplasia (2017) 19, 661–671

Introduction

Despite the availability of a wide variety of therapeutic agents and the success of the addition of the anti-CD20 antibody rituximab to front-line chemotherapy, up to one third of patients with aggressive non-Hodgkin lymphoma (NHL), in particular diffuse large B-cell lymphoma (DLBCL), relapse following initial treatment or are refractory to current therapies. In response to this unmet need, recent efforts have focused on the development of new antibodies to improve overall survival including the characterization of alternative B-cell surface antigens (e.g., CD19, CD22, CD30) that may potentially serve as novel targets for therapeutic intervention [1,2].

One antigen of considerable interest is the tetraspanin CD37, a transmembrane protein proposed to function in immune cell proliferation and survival [3,4]. In normal lymphoid tissues, CD37 shows a restricted expression profile, limited primarily to mature B cells from the pre-B through peripheral stages of differentiation and

Abbreviations: ADC, antibody-drug conjugate; NHL, non-Hodgkin lymphoma; *DLBCL*, diffuse large B-cell lymphoma; ADCC, antibody-dependent cell-mediated cytotoxicity; CDC, complement-dependent cytotoxicity; MMAE, monomethyl auristatin E; SMCC, N-succinimidyl-4-(N-maleimidoethyl) cyclohexane-1-carboxylate; GI, Growth Inhibition; CPM, counts per minute; PR, partial tumor regression; CR, complete response; TFS, tumor free survivors; ABC, activated B-cell; GCB, germinal B-cell.

Address all correspondence to: Stuart W. Hicks, Discovery Research, ImmunoGen Inc., 830 Winter St, Waltham, MA 02451.
E-mail: stuart.hicks@immunogen.com

Received 2 May 2017; Revised 15 June 2017; Accepted 15 June 2017

1476-5586
http://dx.doi.org/10.1016/j.neo.2017.06.001

absent from plasma cells [5,6]. In addition, CD37 is highly expressed on the surface of malignant B cells, including most subtypes of NHL [7,8]. This expression pattern has prompted the development of a number of CD37-targeting therapeutics, including otlertuzumab (TRU-016; a CD37-binding, single-chain homodimeric protein) [9] and BI 836826 (an Fc-engineered antibody) [10], both of which act *via* antibody-dependent cell-mediated cytotoxicity (ADCC) and apoptotic signaling. Each of these agents has now entered clinical testing, but to date, only modest activity has been reported from the initial human trials with otlertuzumab [11,12].

The expression of CD37 on transformed B cells makes it an attractive target for development of antibody-drug conjugate (ADC) therapy. ADCs are designed to deliver chemotherapeutics to tumor cells by linking highly potent small molecules to monoclonal antibodies that recognize tumor-associated antigens, with the goal of improving antitumor activity while reducing systemic toxicity [13]. This approach has been validated in both the hematological and solid tumor settings, as evidenced by FDA approval of two ADCs: brentuximab vedotin (Adcetris), a conjugate of an anti-CD30 antibody with monomethyl auristatin E (MMAE) [14], in relapsed Hodgkin lymphoma and systemic anaplastic large-cell lymphoma, and ado-trastuzumab emtansine (Kadcyla), a conjugate of trastuzumab with the maytansinoid DM1 [15] used to treat patients with HER2-positive metastatic breast cancer. We are developing naratuximab emtansine (IMGN529), an ADC comprised of DM1 linked to a humanized anti-CD37 antibody *via* the same linker used in Kadcyla, as a new investigational agent that effectively combines the intrinsic proapoptotic and effector activities of its antibody component with the cytotoxic activity of the maytansinoid payload [16]. In this manner, IMGN529 can be distinguished from another CD37-targeting ADC, AGS67E, which consists of MMAE linked to an antibody moiety that lacks any intrinsic apoptotic or ADCC activity [17]. IMGN529 binds with high affinity and specificity to CD37, which allow for ADC internalization, processing, and intracellular release of DM1. As a result of its ability to disrupt microtubule assembly, DM1 subsequently induces cell cycle arrest and apoptosis.

IMGN529 has shown robust antitumor activity in preclinical models of CD37-positive NHL [16,18], which led to its clinical evaluation in the treatment of B-cell malignancies. Importantly, the first-in-human phase I trial of IMGN529 conducted in patients with relapsed or refractory B-cell NHL revealed a manageable safety profile and encouraging evidence of single-agent efficacy, particularly in individuals with DLBCL [19]. Here we report that the activity of IMGN529 is significantly enhanced when combined with anti-CD20 antibodies. The findings presented here are of considerable translational relevance for the continued clinical development of IMGN529 as an effective cancer therapeutic.

Materials and Methods

Antibodies and ADC Generation

The humanized anti-CD37 antibody K7135A and nontargeting control IgG of the same isotype and identical Fc sequence were generated at ImmunoGen. The anti-CD19 (huB4) and anti-CD22 (huBU59) antibodies were produced by transient transfection in HEK 293T cells at ImmunoGen. The anti-CD20 antibodies rituximab, ofatumumab, and obinutuzumab were obtained from commercial sources. IMGN529 and the control nontargeting IgG1-SMCC-DM1 conjugate were produced *via* conjugation of the maytansinoid DM1 to the K7153A and the nontargeting IgG1 antibodies, respectively, using the cross-linking agent *N*-succinimidyl-4-(*N*-maleimidoethyl) cyclohexane-1-carboxylate (SMCC) as described previously [16].

Combination Screen

High-throughput combination screens were performed by Horizon CombinatoRx Inc. (Cambridge, MA) to examine IMGN529 activity in combination with 104 compounds. The combination screen was performed in 20 NHL cell lines. Briefly, each cell line was cultured and plated in optimal conditions based on growth characteristics in 384- or 1536-well tissue culture plates. Compounds were added to assay plates using a 6×6 or 6×8 dose matrix of IMGN529 and each combination agent. Concentration ranges were selected based on single agent activity of each molecule on a panel of cell lines. Cell viability was evaluated using an ATPLite assay (Perkin Elmer) following 72 hours of treatment. In addition to IMGN529, the combination activities for K7153A, IgG1-SMCC-DM1 (nontargeting ADC), and unconjugated DM1-Me were determined. All data points were collected *via* automated processes, quality controlled, and analyzed using Horizon CombinatoRx proprietary Chalice software.

Horizon utilizes growth inhibition (GI) as a measure of cell viability. GI is calculated by applying the following test and equation:

$$\text{If } T < V_0 : 100^*(1-(T-V_0)/V_0)$$

$$\text{If } T \geq V_0 : 100^*(1-(T-V_0)/(V-V_0))$$

where T is the signal for a test agent after 72 hours, V is the vehicle-treated control measure at 72 hours, and V_0 is the vehicle control measure at time zero.

A GI reading of 0% represents no growth inhibition where cells treated with compound (T) and (V) vehicle signals are the same. A GI of 100% represents complete growth inhibition or cytostatic conditions where cells treated by compound match the signal of V_0. Compounds reaching GI 200% are considered cytotoxic and represent complete cell death.

To quantitate the combination effects in excess of Loewe additivity, a scalar measure was used to characterize the strength of synergistic interaction termed the Synergy Score.

$$\text{Synergy Score} = \log f_X \log f_Y \Sigma \max(0, \text{Idata})(\text{Idata}-\text{ILoewe})$$

The Synergy Score equation integrates the experimentally observed activity volume at each point in the matrix in excess of a model surface numerically derived from the activity of the component agents using the Loewe model for additivity. Additional terms in the Synergy Score equation are used to normalize for various dilution factors used for individual agents and to allow for comparison of synergy scores across the entire experiment.

Cell Lines

For studies performed at ImmunoGen, the U-2932, SU-DHL-4, DOHH-2, OCI-Ly18, and OCI-Ly7 lines were obtained from DSMZ; the Farage cell line was purchased from ATCC. All lines were characterized by the vendor using routine DNA profiling; no further