MINIREVIEW

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Immune modulation for cancer therapy

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Background: Immune modulation in cancer refers to a range of treatments aimed at harnessing a patient's immune system to achieve tumour control, stabilisation, and potential eradication of disease. A novel therapeutic drug class called immune checkpoint-blocking antibodies modulate T-cell pathways that regulate T cells and have the potential to reinvigorate an antitumour immune response. Ipilimumab was the first FDA-approved immune checkpoint antibody licensed for the treatment of metastatic melanoma (MM) and blocks a checkpoint molecule called cytotoxic T-lymphocyte antigen 4 (CTLA-4).

Methods: Herein we review the preclinical and clinical development of ipilimumab. We outline the mode of action of these agents and other immune checkpoint inhibitors, the management of their toxicities, and how to adequately assess response to treatment.

Results: As a result of these data, a number of other antibodies that block novel checkpoint molecules including programmed death-1 (PD-1), and corresponding ligands such as programmed death ligand-1 (PD-L1) are under preclinical and clinical development, and have demonstrated activity in multiple tumour types.

Conclusions: This review will summarise the mechanism of action and clinical development of immune checkpoint antibodies, as well as lessons learned in the management and assessment of patients receiving these agents.

During immune surveillance, the host provides defense against foreign antigens, while ensuring it limits activation against self-antigens (Page *et al*, 2014). Immune checkpoints are cell surface molecules that serve as endogenous regulators of the immune response, limiting autoimmunity by mediating co-inhibitory signalling pathways (Nirschl and Drake, 2013). In cancer, these pathways are important in the tumour microenvironment and draining lymph nodes, leading to a state of T-cell exhaustion, thereby allowing tumour escape from immune surveillance, and unchecked tumour growth. Monoclonal antibodies (mAbs) that target immune checkpoints either antagonise co-inhibitory immunologic pathways or activate co-stimulatory pathways. These immune checkpoint antibodies are clinically active in a variety of malignancies, including those not traditionally classified as immunogenic, such as non-small-cell lung cancer (NSCLC).

The prototypical immune checkpoint mAb, and first to be approved by the FDA, is ipilimumab (Yervoy, Bristol-Myers Squibb, New York, NY, USA). Ipilimumab is a fully human antibody that targets cytotoxic T-lymphocyte antigen 4 (CTLA-4; Hodi *et al*, 2003) and was approved for metastatic melanoma (MM) based upon two phase III studies demonstrating an improvement in overall survival (OS; Hodi *et al*, 2010; Robert *et al*, 2011). After the initial clinical development of this agent, agents modulating other novel immune checkpoint molecules and their ligands have been identified, such as programmed death-1 (PD-1), programmed death ligand-1 (PD-L1), lymphocyte activation gene-3, T-cell immunoglobulin mucin protein-3, GITR, and CD-137 (Figure 1) among others (Page *et al*, 2014). This article will summarise the underlying mechanisms by which immune checkpoint mAbs elicit an antitumour effect, as well as outline the preclinical and clinical development of both single agent and combination immune modulation with checkpoint antibodies.

ANTI-CTLA-4 ANTIBODIES

Preclinical studies with CTLA-4. CTLA-4 is a molecule expressed on the surface of CD-4 and CD-8 T cells as well as CD25 +

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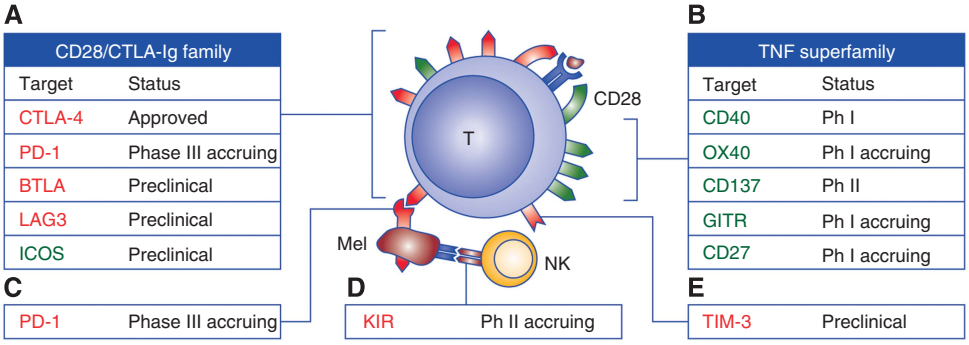

Figure 1. Targets of antibody immune modulators (Page *et al*, 2014). Targets of antibody immune modulators. (**A**) Targetable members of the CD28/CTLA-4 immunoglobulin superfamily include cytotoxic T lymphocyte antigen 4 (CTLA-4), programmed cell death protein 1 (PD-1), B- and T-cell attenuator (BTLA), lymphocyte activation gene 3 (LAG3), and inducible T-cell costimulator (ICOS). (**B**) Targetable members of the tumour necrosis factor (TNF) superfamily include CD40, OX40, CD137/4-1BB, glucocorticoid-induced TNFR-related protein (GITR), and CD27. (**C**) Programmed cell death 1 ligand 1 (PD-L1). (**D**) Killer inhibitory receptor (KIR). (**E**) T-cell Ig and mucin-containing domain 3 (TIM3).

FOXP3+ T-regulatory cells, and is a member of the CD28/immunoglobulin superfamily (Avogadri *et al*, 2011). CTLA-4 competes with CD28 to bind to its ligands B7–1 (CD80) and B7–2 (CD86) on antigen-presenting cells (APCs). CTLA-4 generates an inhibitory signal that blocks a T-cell response. An anti-CTLA-4 antibody was first studied in transplantable tumour models of colon carcinoma (51BLim10), fibrosarcoma (Sa1N and CSA1M), ovarian cancer (OV-HM), and prostate cancer (TRAMPC1; Yang *et al*, 1997). In these models, primary shrinkage of tumours was observed in response to the mAb, and recurrence was not seen at the time of tumour rechallenge.

Anti-CTLA-4 agents: ipilimumab and tremelimumab. Ipilimumab was first tested in MM in a phase I trial of 17 patients and demonstrated two durable partial responses at a starting dose of 3 mg kg^{-1} (Tchekmedyian *et al*, 2002). A dose–response relationship was established in a double-blind phase II trial at three dose levels of 0.3, 3, or 10 mg kg^{-1}, administered every 3 weeks for four doses, followed by 12-weekly maintenance therapy. A response rate (RR) of 11% and a median OS of 14 months were reported in the 10-mg kg^{-1} group, despite a greater incidence of grade 3/4 adverse events (AEs; Wolchok *et al*, 2010). On the basis of these findings, ipilimumab was investigated in the phase III setting in two randomised-controlled trials, that both demonstrated an improvement in OS (Hodi *et al*, 2010; Robert *et al*, 2011). The first trial compared ipilimumab at a dose of 3 mg kg^{-1} with or without gp100 peptide vaccine, *vs* the gp100 peptide vaccine alone (Hodi *et al*, 2010). The median OS in the ipilimumab and ipilimumab/gp100 groups was 10.1 *vs* 10.0 months, respectively, compared with 6.4 months with gp100 alone, with a hazard ratio (HR) of 0.68, $P<0.001$). In the first-line setting, ipilimumab/dacarbazine was compared with dacarbazine/placebo, at an ipilimumab dose of 10 mg kg^{-1}, followed by maintenance ipilimumab or placebo every 12 weeks. This trial demonstrated an improvement in the median OS for the combination arm (11.2 months *vs* 9.1 months; Robert *et al*, 2011). In this study, the number of long-term survivors exceeded the number of patients with objective responses (ORs), with survival rates in the ipilimumab/dacarbazine group at 1 year of (47.3% *vs* 36.3%), 2 years (28.5% *vs* 17.9%), and 3 years (20.8% *vs* 12.2%; HR for death, 0.72; $P<0.001$). Responses were durable, with a median duration of response of 19.3 months with the combination and 8.1 months with dacarbazine alone. These observations suggest that immune-based therapies may generate a sustained antitumour effect in a subset of patients, long after completion of active therapy. Of patients re-induced on the original ipilimumab/gp100 trial, 19% ($n=6/31$) of patients exhibited an OR, with an additional 48% ($n=15/31$) achieving stable disease (SD; Hodi *et al*, 2010). Ipilimumab has also been evaluated in other cancers, with responses observed in both renal cell carcinoma (RCC) and NSCLC (Yang *et al*, 2007; Lynch *et al*, 2012). A three-arm phase II study in NSCLC evaluated concurrent, phased, and sequential ipilimumab combined with carboplatin and paclitaxel in patients with stage IV NSCLC (Lynch *et al*, 2012). This trial demonstrated a benefit in immune-related progression-free survival, defined later in this review. A subset analysis of this study benefit in patients with squamous cell carcinomas of the lung, leading to a large phase III study (NCT01285609).

A second antibody against CTLA-4, tremelimumab (MedImmune LLC, Gaithersburg, MD, USA), is a human immunoglobulin G2 (IgG2) monoclonal antibody (Ribas *et al*, 2007). Ribas *et al* (2013) reported a phase III trial of tremelimumab *vs* investigator's choice of chemotherapy in MM, at a dose of 15 mg kg^{-1} every 3 months. A median duration of response of 36 months was seen with tremelimumab *vs* 14 months with combination chemotherapy ($P=0.0011$); however, no OS benefit was observed. This finding can potentially be explained by the exclusion of patients with an elevated lactate dehydrogenase level, crossover of patients to ipilimumab, as well as possible suboptimal dose and schedule.

ANTI-PD-1/PD-L1 ANTIBODIES

Preclinical studies with the PD-1/PD-L1,2 pathway. The PD-1/PD-L1,2 pathway is a second inhibitory immune checkpoint pathway, mediated by the transmembrane molecule PD-1 (CD279) found on the surface of T lymphocytes, B lymphocytes, and monocytes (Keir *et al*, 2008). PD-1 binds to PD-L1 and PD-L2, which are expressed on APCs and other cells, including tumour and normal tissues. Upon ligation to PD-L1/2, PD-1 suppresses downstream phosphoinositide 3-kinase (PI3K) and Akt signalling via an immunoreceptor tyrosine-based inhibitory motif. In contrast, CTLA-4 signalling inhibits Akt independent of PI3K(-Parry *et al*, 2005). These two pathways therefore bring about similar effects but by different mechanisms.

Anti-PD-1 agents: nivolumab, MK3475 and pidilizumab. The first anti-PD-1 mAb to be clinically evaluated was nivolumab (BMS-936558), a human monoclonal immunoglobulin (Ig) G4 antibody. Results from a Phase Ib study of nivolumab reported activity in MM, RCC and NSCLC (Topalian *et al*, 2012). Objective response rates (ORRs) in these diseases were 28% ($n=26/94$), 24% ($n=4/17$), and 18% ($n=14/76$) respectively. The median duration of response was 74 weeks, with a median OS of 9.6 months in the

Table 1. Current phase III trials investigating antibodies that target the PD-1/PD-L1,2 pathway

Agents	NCI Identifier	Cancer type	Setting	Phase	*n*	Primary end point
Nivolumab *vs* Everolimus	NCT01668784	Pre-treated or metastatic RCC	Pre-treated or first-line	III	822	Overall survival
Nivolumab *vs* Nivolumab + Ipilimumab *vs* Ipilumab	NCT01844505	Metastatic melanoma	First-line	III	917	Overall survival
Nivolumab *vs* Dacarbazine	NCT01721772	Metastatic melanoma	First-line	III	410	Overall survival
MK-3475 (2 dose levels) *vs* Ipilimumab	NCT01866319	Metastatic melanoma	Pre-treated or first-line	III	645	Overall survival Progression-free survival
MPDL3208A	NCT01846416	NSCLC (PDL1 +)	First-line	II	130	Response rate
MPDL3280A *vs* Docetaxel	NCT01903993	NSCLC	Platinum pre-treated	II	180	Response rate

Abbreviations: NCI = National Cancer Institute; NSCLC = non-small cell lung cancer; RCC = renal cell carcinoma.

patients with heavily-pretreated NSCLC. Nivolumab monotherapy at a dose of 3 mg kg^{-1} is currently being studied in phase III clinical trials in MM, RCC and NSCLC (Table 1). Successful re-induction with nivolumab has been described in a patient with melanoma who achieved a partial response (PR), followed by a period of SD for 16 months off treatment (Lipson *et al*, 2013).

A second humanised monoclonal IgG4 anti-PD-1 antibody, MK-3475 (Merck, Sharpe and Dohme, Whitehouse Station, NJ, USA), was deemed safe at 1 mg kg^{-1}, 3 mg kg^{-1}, and 10 mg kg^{-1} dose levels administered every 2 weeks in a phase I study, with no maximum tolerated dose (MTD) identified (Patnaik and Tolcher, 2012). This agent was subsequently studied in both first-line and ipilimumab-pretreated patients with MM, at dose levels 2 and 10 mg kg^{-1} administered every 2 or 3 weeks. Identical immune-related responses by immune-related response criteria (irRC), detailed later in this review, were 56% in both first-line and ipilImumab-pretreated patients at 10 mg kg^{-1} given every 2 weeks (Hamid *et al*, 2013). A phase II trial of this agent at two dose levels in comparison to chemotherapy, is currently in accrual (NCT01704287), as well as a phase III study in advanced MM compared to ipilimumab (NCT01866319).

In hematologic malignancies, the anti-PD-1 antibody pidilizumab (CT-011) demonstrated a ORR of 66% ($n = 19/32$) when combined with rituximab in relapsed follicular lymphoma (Westin *et al*, 2014). An ORR of 51% was also seen in a phase II study of 35 patients with measurable disease after autologous stem-cell transplant for diffuse large B-cell lymphoma ($n = 18/35$) (Armand *et al*, 2013). This agent is currently being studied in MM, malignant gliomas, RCC and combination with chemotherapy in advanced colorectal cancer (NCT01435369, NCT01952769, NCT01441765, NCT00890305).

Anti-PD-L1 agents: BMS-936559 and MPDL3280A. The first anti-PD-L1 mAb to be clinically evaluated was BMS-936559. Brahmer and colleagues reported a phase I study of this agent in multiple tumour types, including 75 patients with NSCLC and 52 with MM (Brahmer *et al*, 2012). The ORR 17% of patients with MM was 17% ($n = 9/52$), 12% in RCC ($n = 2/17$), 10% in patients with NSCLC ($n = 5/49$). No MTD was found, and 9% of patients sustained grade 3/4 AEs. MPDL3280A is a second human mAb against PD-L1, and contains an engineered Fc portion that targets PD-L1. In a phase I trial of MPDL3280A administered to patients with MM, RCC and NSCLC, RR's were 29%, 22% and 15% respectively (Hamid and Lawrence, 2012). Table 1 outlines current phase III trials with nivolumab and other agents targeting the PD-1/PD-L1,2 pathway, in a number of tumour types. Additional PD-L1-directed agents such as MEDI-4736 are being evaluated in the phase I setting.

TOXICITIES WITH IMMUNE CHECKPOINT ANTIBODIES

The spectrum of AEs associated with immune checkpoint antibodies are termed immune-related AEs (irAE's). The underlying pathophysiology relates to the immune-based mode of action of these agents, leading to T-cell inflammatory infiltration of solid organs, and increased serum inflammatory cytokines (Fecher *et al*, 2013). irAEs observed with ipilumumab appear to be dose-dependent, and range in severity from mild to fatal, and in onset from slow to sudden, but generally appear late, 8–10 weeks after initiation of therapy (Weber *et al*, 2012). Medical management is aimed at correctly identifying the irAE, grading based on the National Cancer Institute common toxicity criteria of AEs, and initiating early treatment with supportive care or immunosuppressive medications, typically corticosteroids. AEs with ipilumumab and tremelimab were common and ranged from mild to severe, with the incidence of grade 3/4 irAEs in phase III studies ranging from 17 to 56% (Ribas *et al*, 2007; Hodi *et al*, 2010; Robert *et al*, 2011). The most commonly observed irAEs with ipilumumab and tremelimumab are dermatitis (pruritus, rash), enterocolitis, endocrinopathies (hypophysitis, thyroiditis), liver enzyme abnormalities, and uveitis (Page *et al*, 2014). irAEs including mild fatigue, rash, diarrhea, and colitis have been described with anti-PD-1 agents, although AEs seem to be less common than those seen with anti-CTLA-4 antibodies (Gangadhar and Vonderheide, 2014). A rare but potentially fatal inflammatory pneumonitis was observed in 2–4% of cases, and led to three treatment-related deaths in the original phase I study of nivolumab (Topalian *et al*, 2012). In the phase I study of MK-3475 in MM, 13% of patients developed grade 3/4 irAEs, and 4% developed mild (grades 1–2) pneumonitis (Hamid *et al*, 2013). MPDL3280A had a 30–40% incidence of grade 3/4 AEs including hyperglycaemia, nephritis, and fatigue, but no cases of grade 3–5 pneumonitis were reported (Hamid and Lawrence, 2012). Toxicities may vary across tumour types, possibly explained by heterogeneity of antigen expression and resulting autoreactivity.

Management algorithms have emerged for irAE's, such as inflammatory diarrhea/colitis and uniformly begin with elimination of potential infectious etiologies. Grade I diarrhea is managed with oral hydration, an American Dietary Association colitis diet, and loperamide (Weber *et al*, 2012). Diarrhea of grade 2 or greater can be managed with oral budesonide, oral corticosteroids, intravenous methylprednisolone, and occasionally infliximab (Fecher *et al*, 2013). Colitis-associated mortality is associated with management delays, failure to withhold ipilimumab, and an inadequate antidiarrheal regimen. The use of effective management algorithms have reduced life-threatening complications, with bowel perforations now occurring in $<1\%$ of patients.

ASSESSING RESPONSE TO IMMUNOTHERAPEUTICS

Antitumour responses with immunotherapies are heterogenous: responses may be mixed or delayed, lesions may enlarge before shrinking, lesions may remain stable or slowly regress over time. These responses can be potentially explained by T-cell activation and tumoral infiltration by immune cells, as well as intra-patient heterogeneity of tumour–host interactions. These observations led a group of colleagues to propose the 'immune-related response criteria (irRC)' for evaluating response to immunotherapeutic agents. These criteria are based on the rationale that immunotherapies generate an antitumour effect with response kinetics distinct from cytotoxic chemotherapy (Wolchok *et al*, 2009). The irRC thus recommends interval imaging at least 4 weeks apart to aid in the confirmation of asymptomatic progression. Owing to these response patterns, patients who experience clinically insignificant progression often continue therapy until progression is confirmed on subsequent imaging.

Intriguing clinical effects with immune checkpoint antibodies and radiation therapy have also been observed. The abscopal effect refers to tumour regression occurring outside an irradiated field, when a patient is receiving immunomodulatory therapy (Postow *et al*, 2012). The underlying mechanism is thought to be due to the stimulation of antigen release by local inflammation at the site of radiotherapy. The combination of radiation plus immunotherapy is being prospectively studied (NCT01703507, NCT01497808, NCT01565837, and NCT01689974).

BIOMARKERS TO IMMUNE CHECKPOINT MOLECULES

As only a subset of patients treated with immune checkpoint antibodies experience durable and long-term disease control, predictive biomarker development has become a priority. The absolute lymphocyte count (ALC) is a potential pharmacodynamic biomarker for ipilimumab, with the ALC at 7 weeks ($\geqslant 1000$ cells μl^{-1}) and magnitude of ALC increases with therapy demonstrating promising results (Ku *et al*, 2010; Postow and Panageas, 2012). Baseline absolute eosinophil count and relative eosinophil count have also been associated with improved survival in these patients (Delyon *et al*, 2013; Schindler and Postow, 2013).

Potential predictive biomarkers for PD-1/PD-L1 targeting agents include PD-L1 expression by tumour cells by a variety of immunohistochemical (IHC) techniques and laboratory assays. Patients with tumours expressing PD-L1 in the original phase I trial of nivolumab had an ORR of 44% *vs* 17% among PD-L1-negative patients (Topalian *et al*, 2012). However, PD-L1 expression in a recent ipilimumab + nivolumab trial revealed similar response in both PD-L1-positive and -negative groups (RR $n = 8/17$ in PD-L1 negative *vs* $n = 4/10$ in PD-L1 positive; Grosso and Inzunza, 2013). Variations in IHC technique, cancer type, primary *vs* metastatic lesions, and treatment history are likely to contribute to PD-L1 expression. In addition, PD-L1 expression has been shown to be dynamic, and associated with tumour-intrinsic and tumour-extrinsic factors, such as loss of PTEN tumour suppressor expression, as well as interferon gamma production (Callahan and Curran, 2013). PD-L1 expression is being prospectively evaluated as a potential predictive biomarker in a phase III trial comparing nivolumab *vs* chemotherapy in melanoma (NCT01721746). It is important to recognise that no study to date has shown a 0% RR in patients with PD-L1-negative tumours, implying that this is not a binary indicator of potential durable benefit. Careful consideration should be exercised before any treatment decisions are made based on a heterogeneously and dynamically inducible biomarker. This is very much unlike static genetically encoded biomarkers, such as BRAF or EGFR mutations.

COMBINATORIAL APPROACHES

The combination of ipilimumab and nivolumab was recently evaluated in a phase I trial in MM, demonstrating a RR of 53% at the MTD, with all responding subjects in this cohort achieving a $\geqslant 80\%$ decline in tumour burden at 12 weeks (Wolchok *et al*, 2013). The combination was safe; however, there were more frequent (53%) grade 3/4 AEs than with either agent as monotherapy. Other mAb combinations are currently being investigated, and are detailed in Table 2. Another strategy is to combine checkpoint agents with other standard or investigational anticancer therapies including radiotherapy, cytotoxic chemotherapy, targeted therapies, or vaccine/cytokine therapy. However, the combination of ipilimumab + BRAF inhibitor vemurafenib produced significant hepatotoxicity, requiring termination of a phase I trial. (Ribas *et al*, 2013) A study of dabrafenib and trametinib with ipilimumab is ongoing and trials of targeted therapies with PD-1 pathway-blocking drugs are about to be initiated in MM.

Table 2. Current clinical trials investigating combinations of immune checkpoint inhibitors

Agents	NCI Identifier	Cancer Type	Phase	*n*	Primary end points
MEDI4736 + Tremelimimab	NCT02000947	NSCLC	Ib	156	Safety MTD
Anti-LAG-3 (BMS986016) + Nivolumab	NCT01968109	Multiple solid tumours	I	168	Safety MTD
Anti-IL2 (BMS982470) + Nivolumab	NCT01629758	Multiple solid tumours	I	165	Safety MTD
Anti-Kir (Lirilumab) + Nivolumab	NCT01714739	Multiple solid tumours	I	150	Safety MTD
Nivolumab *vs* Nivolumab + Ipilumumab	NCT01928394	Gastric SCLC Pancreatic Triple negative breast cancer	I/II	160	Objective response rate
Nivolumab + Sequential Ipilumumab	NCT01783938	Metastatic melanoma	II	100	Safety

Abbreviations: GEJ = gastroesophageal junction; KIR = killer-cell immunoglobulin-like receptor; LAG-3 = Lymphocyte-activation gene 3; MTD = maximum tolerated dose; NCI = National Cancer Institute; NSCLC = non-small cell lung carcinoma; SCLC = small cell lung carcinoma; TNBC = triple negative breast cancer.

NOVEL IMMUNE CHECKPOINT MOLECULES

New agents that attempt to target other immunomodulatory receptors on T cells and other immune cells are in development (Figure 1). Agonists of co-stimulatory molecules on B and T cells such as CD-137, OX40, and glucocorticoid-induced TNFR-related protein (GITR) are in clinical development (Mallett *et al*, 1990). A phase I study of an OX40 agonist in advanced solid tumours demonstrated tumour shrinkage of at least one metastatic lesion in 12 out of 30 subjects after one cycle of therapy (Curti *et al*, 2013). A humanised anti-GITR mAb (TRX518) also enhances co-stimulation in human lymphocytes *in vitro*, and is being studied in a dose-escalation trial (NCT1239134). CD-137/4-1BB is a third co-stimulatory mediator present on activated T cells, with corresponding ligands on activated B cells, and APCs (Lin *et al*, 2008). Agonist mAbs for CD137 enhance the co-stimulatory signal on T cells and such mAbs against CD137 have entered clinical trials in haematologic malignancies and others (urelumab: NCT01471210, NCT01775631; PF-05082566: NCT01307267).

CONCLUSION

Preliminary studies of CTLA-4, PD-1, and PD-L1-blocking antibodies show clear evidence of clinical activity, proving that immune checkpoint modulation is a viable emerging treatment modality across malignancy types, even in cancers not traditionally viewed as amenable to immunotherapy. However, because responses are confined to a subset of treated subjects, future development will focus upon rational combinatorial approaches and predictive biomarker discovery.

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