L-BLP25: A Peptide Vaccine Strategy in Non–Small Cell Lung Cancer

Randeep Sangha and Charles Butts

Abstract MUC1 is a mucinous glycoprotein which is overexpressed and under or aberrantly glycosylated in many human malignancies. MUC1 is associated with cellular transformation and can confer resistance to genotoxic agents. L-BLP25 is a peptide vaccine strategy that targets the exposed core peptide of MUC1. In preclinical studies, L-BLP25 induced a cellular immune response characterized by T-cell proliferation in response to MUC1 and production of IFN-γ. Phase I and II trials have established the dose and schedule of the vaccine as well as its excellent safety profile. A randomized phase II trial of maintenance L-BLP25 versus best supportive care in patients with stage IIIB/IV non–small cell lung cancer who experienced clinical benefit from initial therapy has been reported. Updated survival analysis of this trial continues to show a strong survival trend in favor of L-BLP25 (median survival, 30.6 versus 13.3 months) in a subgroup of patients with locoregional stage IIIB disease. These promising results will be tested in a phase III trial of L-BLP25 versus placebo in patients with stage III non–small cell lung cancer after response to primary chemoradiotherapy.

The identification of tumor-associated antigens has triggered an interest in cancer immunotherapy as a therapeutic option. The mucinous transmembrane glycoprotein MUC1 is widely distributed in normal and abnormal tissues. MUC1 is a heavily glycosylated integral membrane protein normally restricted to the apical surface of polarized epithelial cells, including those of the respiratory tract (1). At the biochemical level, MUC1 has a large NH_2-terminal ectodomain with five potential *O*-glycosylation sites at each of its 20-amino acid–long tandem repeats (2, 3). The COOH-terminal domain contains a short extracellular region, a transmembrane anchoring domain, and a 72-amino acid cytoplasmic tail.

In many epithelial malignancies, MUC1 is overexpressed and loses its polarity of expression (4). Strikingly, the NH_2-terminal ectodomain becomes under or aberrantly glycosylated, with shortened carbohydrate side chains, unmasking epitopes on its peptide core. The truncated carbohydrate chains can act as tumor-associated neo-epitopes (Tn, sialyl-Tn) and, as a result of incomplete glycosylation, the variable number tandem repeat regions of the peptide backbone are also exposed to a potential cellular and humoral immune response.

Role of MUC1

The precise function of the MUC1 molecule remains undefined in normal and malignant cells, although certain insights are available regarding the role of MUC1 in promoting tumor cell growth and survival. Recent studies suggest that MUC1 is involved in tumorigenicity, tumor cell migration, and resistance to stress-induced apoptosis and to chemotherapeutic agents (3, 5–10).

Activated epidermal growth factor receptor regulates the interaction of MUC1 with c-Src tyrosine kinase and β-catenin (3). Using ZR-75-1 breast carcinoma cells, Li et al. showed that the MUC1 cytoplasmic domain is phosphorylated by activated epidermal growth factor receptor, which leads to binding of MUC1 to c-Src and increased binding to β-catenin. Overexpression of MUC1 in human carcinoma cells possibly contributes to the transformed phenotype by dysregulating these interactions. MUC1 cytoplasmic domain binds directly to β-catenin and blocks glycogen synthase kinase 3β–mediated phosphorylation and subsequent degradation of β-catenin (5). The resulting elevated levels of stabilized, dephosphorylated, β-catenin contribute to MUC1 tumorigenicity.

Tumor-associated MUC1 interacts with intercellular adhesion molecule-1 by triggering a migratory signal (6). The depolarized membrane distribution of MUC1 may facilitate interaction with stromal/endothelial intercellular adhesion molecule-1, thereby promoting cell adhesion and subsequent migration of tumor cells through the vasculature and fibroblast-containing stroma.

Overexpression of MUC1 by carcinoma cells may confer a survival advantage under conditions of oxidative or other forms of stress (7). Oxidative stress up-regulates MUC1 expression by activating *MUC1* gene transcription. The increased expression of MUC1 attenuates the intracellular levels of reactive oxygen species and inhibits the apoptotic response to oxidative stress. Yin et al. showed that the reactive oxygen species–induced apoptotic response can be attenuated by MUC1 cytoplasmic domain regulation of the FKHRL1/FOXO3a signaling pathway (8). FKHRL1/FOXO3a is a member of the forkhead family of transcription factors that stimulates oxidant scavenging and DNA repair. In addition, the MUC1 cytoplasmic domain associates directly with the tumor suppressor p53, particularly

Authors' Affiliation: Cross Cancer Institute, Edmonton, Alberta, Canada
Received 1/25/07; revised 4/23/07; accepted 5/8/07.
Presented at the Fourth Cambridge Conference on Novel Agents in the Treatment of Lung Cancer, Cambridge, Massachusetts, September 29-30, 2006.
Requests for reprints: Charles Butts, Department of Medical Oncology, Cross Cancer Institute, 11560 University Avenue, Edmonton, Alberta, Canada, T6G 1H7. Phone: 780-432-8513; Fax: 780-432-8888; E-mail: charlesb@cancerboard.ab.ca.

doi:10.1158/1078-0432.CCR-07-0213

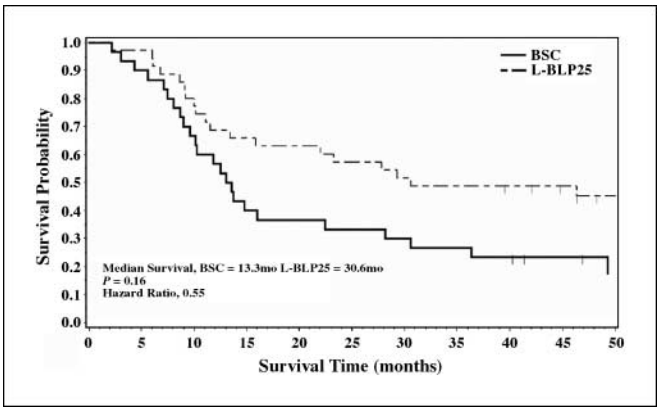

Fig. 1. Updated survival analysis, with median follow-up of 53 mo, for stage IIIB locoregional patients. Median survival 30.6 mo (mo) for L-BLP25 – treated patients, and 13.3 mo for best supportive care (*BSC*).

after genotoxic stress (9). This interaction obstructs the p53-dependent apoptotic response to DNA damage.

Finally, MUC1 has been shown to localize to the mitochondria and block activation of the intrinsic apoptotic pathway by DNA-damaging agents (10). This represents a mechanism that seems to confer resistance to certain chemotherapeutic agents.

MUC1 Immunosuppression

High levels of serum MUC1 are associated with poor prognosis and immunosuppression in patients with advanced adenocarcinoma (11). MUC1 purified from ascitic fluid of cancer patients suppresses T-cell proliferative response (12). Hiltbold et al. showed that there is a failure to process and present soluble MUC1 on class II MHC, although it is readily taken up by dendritic cells (13). Conceivably, cells overexpressing tumor-associated MUC1 might escape a strong host immune response through this mechanism.

Indeed, the multiple roles of MUC1 in cellular transformation, tumor cell migration, chemoresistance, and immunosuppression make this glycoprotein an attractive target for cancer immunotherapy.

Liposomal MUC1 Vaccine BLP25 (L-BLP25)

BLP25 liposome vaccine (L-BLP25) is a cancer vaccine strategy that targets the exposed core peptide of MUC1 tumor-associated antigen. The BLP25 lipopeptide consists of a 25-amino acid sequence (STAPPAHGVTSAPDTRPAPGSTAPP) that provides MUC1 specificity. It is slightly larger than one tandem repeat and contains a palmitoyl lysine residue at the carboxy terminal to enhance the incorporation of the lipopeptide into the liposome particle The vaccine is a lyophilized preparation consisting of BLP25 lipopeptide, immunoadjuvant monophosphoryl lipid A, and three lipids (cholesterol, dimyristoyl phosphatidylglycerol, and dipalmitoyl phosphatidylcholine). Alternative MUC1 vaccine strategies have used dendritic cells pulsed with MUC1 antigens or a recombinant MVA-MUC1-IL-2 vaccinia vector (modified vaccinia Ankara) expressing MUC1 and IL-2 (14, 15).

The liposomal delivery system presents a novel approach to target MUC1. It is believed to facilitate uptake by antigen-presenting cells such that the lipopeptide is delivered into the intracellular space for presentation by MHC molecules.

Preclinical and Clinical L-BLP25 Studies

In preclinical murine studies, L-BLP25 induced a cellular immune response characterized by antigen-specific T-cell proliferation and production of IFN-γ (16, 17). Ultimately, this led to early phase I and II clinical trials to assess its safety profile and efficacy. The clinical development of L-BLP25 has been pursued primarily in previously treated patients with advanced non – small cell lung cancer (NSCLC). A pilot phase II study in patients with prostate cancer has recently been conducted (18).

An initial phase I study in patients with NSCLC showed that the vaccine could be administered with minimal toxicity (19). Two phase II trials established the dose and schedule of L-BLP25 and showed the capability of the vaccine in eliciting a T-cell proliferative response (20). The survival in those patients with advanced NSCLC, who received L-BLP25, was sufficiently encouraging to proceed with a phase II randomized study.

Randomized Phase II Study in NSCLC

In order to test the efficacy of L-BLP25 in advanced NSCLC, an open-label randomized phase II trial was undertaken (21). Patients with stable disease or responding stage IIIB or IV NSCLC after any first-line chemotherapy were randomly assigned to either L-BLP25 plus best supportive care or best supportive care alone. Patients in the L-BLP25 arm received a single i.v. dose of cyclophosphamide (300 mg/m^2) followed by eight weekly s.c. immunizations of L-BLP25 (1,000 μg). Subsequent immunizations were administered at 6-week intervals.

The study was powered to detect a difference in survival of 5 months, with a power of 80%. This was an ambitious targeted treatment benefit, but suitable for a randomized phase II trial with a restricted sample size. As previously reported, this trial enrolled 171 patients from 17 centers in Canada and the United Kingdom. Overall survival was 17.4 months for L-BLP25 – treated patients and 13.0 months for patients assigned to best supportive care (P = 0.065). A post hoc subgroup analysis of 65 patients with stage IIIB locoregional disease (IIIB-LR) suggested that the difference in survival with the vaccine was confined to this group. At the time of the original report, median survival in patients with stage IIIB locoregional disease who were treated with L-BLP25 had not been reached, whereas median survival for the patients assigned to best supportive care was 13.3 months. Updated survival analysis of this trial[1] with median follow-up of 53 months, continues to show a strong trend in favor of L-BLP25 – treated patients with stage IIIB disease (median survival, 30.6 versus 13.3 months; P = 0.16; Fig. 1). Although this represents a subgroup analysis with a nonsignificant P value, the magnitude of the difference and its durability over time warrant further study.

[1] C. Butts et al., submitted for publication.

Future Directions

Based on the encouraging results seen in the randomized phase II trial, an international, randomized phase III trial of L-BLP25 vaccine versus placebo in patients with stage III NSCLC has begun. Additional rationales for selecting this patient population include the lack of any currently approved standard maintenance therapy and the lower tumor burden in this setting, which theoretically would result in a lower likelihood of immune resistance. This trial enrolls patients with stage IIIA or IIIB NSCLC who have completed first-line treatment with chemoradiation, either concurrently or sequentially, and who have shown stable disease or an objective clinical response. The Stimulating Targeted Antigenic Responses to NSCLC trial, which opened in February 2007, will test the hypothesis that L-BLP25 vaccine alters the survival of patients with stage III NSCLC who have received treatment with curative intent.

Open Discussion

Dr. Thomas Lynch: How many of us are skeptical that immune therapy will ever pan out in non–small cell lung cancer? Show of hands. So we have a skeptical crowd. I would argue that this randomized phase II study was well done. It has been interpreted it in the right way. The data have not been oversold, as was done with other immune compounds in the past.

Dr. Bruce Johnson: I am skeptical. I hope I am wrong. We commonly see that a new immunological compound does not work in the whole group, it works in a subset. It may or may not have been a planned subset, and appropriately, this finding generates additional hypotheses. Then, when it is tested in that subset, historically, it does not work for the specific subgroup. It would be helpful to understand why this would work in stage IIIB and not in the other patients.

Dr. Butts: Some of the negative trials involved poor selection of patients. It takes at least 8 weeks just to deliver the vaccine. If you are doing that in patients with metastatic or refractory non–small cell lung cancer, you don't have time to show a difference. In terms of why it would work in stage III versus stage IV disease, immune resistance mechanisms are much more active in advanced disease. We have learned more about these mechanisms of immune resistance, and many of the vaccine strategies are trying to incorporate ways to get around them. Cyclophosphamide, for example, may inhibit suppressor T-cell function.

Dr. Angela Davies: While I am one of the skeptics, patients love these studies. The concept of stimulating your immune system is why they go to the naturopath, the Chinese herbal store, because they love that concept that they are fighting their cancer with their own immune system. Accrual is going to be very successful; whether it will work or not is another issue.

Dr. Butts: We had to do a phase II trial to demonstrate that that adjuvant did not produce undue toxicity. We did it in the stage III setting with five or six centers in Canada accruing 22 patients in about 6 months. I rarely have a patient say, "I'm not interested in the vaccine trial."

Dr. Johnson: If you get your chest, including the thymus, irradiated, that could be good, it could be bad, and it would be nice to have an explanation. Do you have some idea about whether irradiating a person's chest helps or hurts the vaccine?

Dr. Butts: We don't have any specific data. A number of these stage III patients did get radiation, and that is where we see the survival signal. There is some potential for an enhancement to the immune response because when you get tumor kill, you get release of new antigens and so there is antigen spreading within the immune system. So, if you stimulate response to MUC1, you may actually get cross-stimulation to other antigens that are released after you treat the tumor.

References

1. Vlad AM, Kettel JC, Alajez NM, Carlos CA, Finn OJ. MUC1 immunobiology: from discovery to clinical applications. Adv Immunol 2004;82:249–93.
2. Karsten U, Mensdorff-Pouilly S, Goletz S. What makes MUC1 a tumor antigen? Tumor Biol 2005;26:217–20.
3. Li Y, Ren J, Yu W, et al. The epidermal growth factor receptor regulates interaction of the human DF3/MUC1 carcinoma antigen with c-Src and β-catenin. J Biol Chem 2001;276:35239–42.
4. Ho SB, Niehans GA, Lyfgot C, et al. Heterogeneity of mucin gene expression in normal and neoplastic tissues. Cancer Res 1993;53:652–7.
5. Huang L, Chen D, Liu D, Yin L, Kharbanda S, Kufe D. MUC1 oncoprotein blocks glycogen synthase kinase 3β-mediated phosphorylation and degradation of β-catenin. Cancer Res 2005;65:10413–22.
6. Rahn JJ, Chow JW, Horne GJ, et al. MUC1 mediates transendothelial migration *in vitro* by ligating endothelial cell ICAM-1. Clin Exp Metastasis 2005;22:475–83.
7. Yin L, Li Y, Ren J, Kuwahara H, Kufe D. Human MUC1 carcinoma antigen regulates intracellular oxidant levels and the apoptotic response to oxidative stress. J Biol Chem 2003;278:35458–64.
8. Yin L, Huang L, Kufe D. MUC1 oncoprotein activates the FOXO3a transcription factor in a survival response to oxidative stress. J Biol Chem 2004;279:45721–7.
9. Wei X, Xu H, Kufe D. Human MUC1 oncoprotein regulates p53-responsive gene transcription in the genotoxic stress response. Cancer Cell 2005;7:167–78.
10. Ren J, Agata N, Chen D, et al. Human MUC1 carcinoma-associated protein confers resistance to genotoxic anticancer agents. Cancer Cell 2004;5:163–75.
11. Reddish MA, MacLean GD, Poppema S, Berg A, Longenecker BM. Pre-immunotherapy serum CA27.29 (MUC-1) mucin level and $CD69^+$ lymphocytes correlate with effects of theratope sialyl-Tn-KLH cancer vaccine in active specific immunotherapy. Cancer Immunol Immunother 1996;42:303–9.
12. Agrawal B, Krantz MJ, Reddish MA, Longenecker BM. Cancer-associated MUC1 mucin inhibits human T-cell proliferation, which is reversible by IL-2. Nat Med 1998;4:43–9.
13. Hiltbold EM, Vlad AM, Cibrowski P, Watkins SC, Finn OJ. The mechanism of unresponsiveness to circulating tumor antigen MUC1 is a block in intracellular sorting and processing by dendritic cells. J Immunol 2000;165:3730–41.
14. Kontani K, Taguchi O, Ozaki Y, et al. Dendritic cell vaccine immunotherapy of cancer targeting MUC1 mucin. Int J Mol Med 2003;12:493–02.
15. Squiban P, Velu T, Mennecier M, et al. MVA-MUC1–2 vaccine immunotherapy for advanced non small cell lung cancer (NSCLC): interim phase II data [2004 Annual Meeting Proceedings Post-Meeting Edition]. J Clin Oncol 2004;22:2544.
16. Agrawal B, Krantz MJ, Reddish MA, Longenecker BM. Rapid induction of primary human CD4+ and CD8+ T cell responses against cancer-associated MUC1 peptide epitopes. Int Immunol 1998;10:1907–16.
17. Guan HH, Budzynski W, Koganty RR, et al. Liposomal formulations of synthetic MUC1 peptides: effects of encapsulation versus surface display of peptides on immune responses. Bioconjug Chem 1998;9:451–8.
18. North SA, Graham K, Bodnar D, Venner P. A pilot study of the liposomal MUC1 vaccine BLP25 in prostate specific antigen failures after radical prostatectomy. J Urol 2006;176:91–5.
19. Palmer M, Parker J, Modi S, et al. Phase I study of the BLP25 (MUC1 peptide) liposomal vaccine for active specific immunotherapy in stage IIIB/IV non-small-cell lung cancer. Clin Lung Cancer 2001;3:49–57.
20. North S, Butts C. Vaccination with BLP25 liposome vaccine to treat non-small cell lung and prostate cancers. Expert Rev Vaccines 2005;4:249–57.
21. Butts C, Murray N, Maksymiuk A, et al. Randomized phase IIB trial of BLP25 liposome vaccine in stage IIIB and IV non-small-cell lung cancer. J Clin Oncol 2005;23:6674–81.

Clinical Cancer Research

L-BLP25: A Peptide Vaccine Strategy in Non–Small Cell Lung Cancer

Randeep Sangha and Charles Butts

Clin Cancer Res 2007;13:4652s-4654s.