OncoImmunology 2:10, e25989; October 2013; © 2013 Landes Bioscience

Combining cellular and gene therapy approaches for treatment of intracranial tumors

Michelle J Hickey[1], Noriyuki Kasahara[2,3], Barbara M Mueller[4], and Carol A Kruse[1,*]

[1]Department of Neurosurgery; University of California Los Angeles; Los Angeles CA USA;
[2]Department of Medicine; University of California Los Angeles; Los Angeles CA USA; [3]Department of Molecular and Medical Pharmacology; University of California Los Angeles; Los Angeles, CA USA; [4]Torrey Pines Institute for Molecular Studies; San Diego, CA USA

Keywords: breast cancer, brain cancer, metastasis, gene therapy, immunotherapy, alloCTL, retrovirus

New treatments are needed for brain metastasis, which is associated with high morbidity and mortality. Two novel cellular and gene therapy modalities were evaluated in xenograft models for human breast cancer. The individual and especially the combined treatments with alloreactive cytotoxic T lymphocytes and replicating retroviral vectors coding for prodrug activating enzymes followed later with nontoxic prodrug demonstrated efficacy without off-target effects.

Metastasis to the brain is a late step in the progression of many solid tumors including breast cancer, lung cancer and melanoma.[1,2] In the clinic brain metastases are more frequently seen in recent years,[3] a fact that is thought to be due to advances in the systemic treatment of the underlying malignancy (for example in breast cancer) and also to increasing rates of some tumors such as malignant melanoma. Brain metastases diminish quality of life and shorten survival of patients with advanced cancer.[1–4] The biology of brain metastases, which includes the fact that they often present as multiple foci in intracerebral sites, and their relative protection from systemic therapies by the blood-brain barrier, present serious challenges to therapy. Currently, the treatment of brain metastases is similar to that for primary brain tumors, mainly radiotherapy and neurosurgical resection.[3,4] These treatments are more palliative than curative. New, effective therapies are sorely needed to improve patient outcomes.

We are exploring targeted therapies for the treatment of brain metastases in preclinical models. One treatment modality that we view as very promising is cellular immunotherapy using alloreactive cytotoxic T lymphocytes (alloCTL). AlloCTL, lymphocytes from unrelated blood donors that are sensitized to the human leukocyte antigens (HLA) of the tumor-bearing host, target brain cancer cells because they display HLA whereas normal neuroglia do not. AlloCTL have demonstrated in vitro and in vivo promise in preclinical glioma model studies[5] and are currently being tested in a Phase I dose escalation trial in recurrent glioma (NCT01144247; www.clinicaltrials.gov). A different treatment modality for glioma uses replicating retroviral vectors (RRV) to deliver a prodrug activating enzyme that when followed with prodrug has shown beneficial preclinical results.[6,7] The therapeutic RRV encode yeast cytosine deaminase (RRV-CD) that activates the prodrug 5-fluorocytosine (5FC) into toxic 5-fluorouracil (5FU). In quiescent brain, RRV preferentially infects dividing brain cancer cells. RRV with prodrug is currently being explored in the clinic as an experimental treatment for glioma (NCT01156584 and NCT01470794; www.clinicaltrials.gov). Recently, we assessed the therapeutic efficacy of alloCTL and RRV used individually or in combination in xenograft models of human breast cancer.[8]

To generate alloCTL that recognize and attack the MDA-MB-231 (231) human breast tumor cell line, peripheral blood mononuclear cells were isolated from healthy, HLA-mismatched donors and combined with gamma-irradiated 231 cells in a one-way mixed lymphocyte tumor reaction. The resulting alloCTL were mainly $CD3^+/CD8^+$ T cells that proliferated and produced proinflammatory IFNγ when coincubated with breast cancer targets and displayed potent cytotoxicity against 231 cells and the brain seeking subline 231BR in vitro. In immune deficient mice, alloCTL were placed into a 231BR tumor focus, induced tumor cell apoptosis and trafficked toward another established tumor focus in contralateral brain, where they also caused tumor cell injury.[8]

The efficacies of individual and combined alloCTL and RRV therapies were tested in subcutaneous and intracranial established 231 tumor xenograft models in immune deficient mice. Subcutaneous tumor growth was significantly reduced in alloCTL and gene therapy treated groups compared with controls, with the largest reduction in tumor volume observed in mice treated with both alloCTL and RRV-CD + 5FC. In an intracranial model, we show that RRV efficiently transduce 231BR intracranial tumors without detectable spread to normal brain. Further, mice with established intracranial 231BR tumors treated with combined alloCTL

*Correspondence to: Carol A Kruse; Email: ckruse@mednet.ucla.edu
Submitted: 07/29/2013; Accepted: 07/31/2013
Citation: Hickey MJ, Kasahara N, Mueller BM, Kruse CA. Combining cellular and genetic approaches for the treatment of intracranial tumors. OncoImmunology 2013; 2:e25989; http://dx.doi.org/10.4161/onci.25989

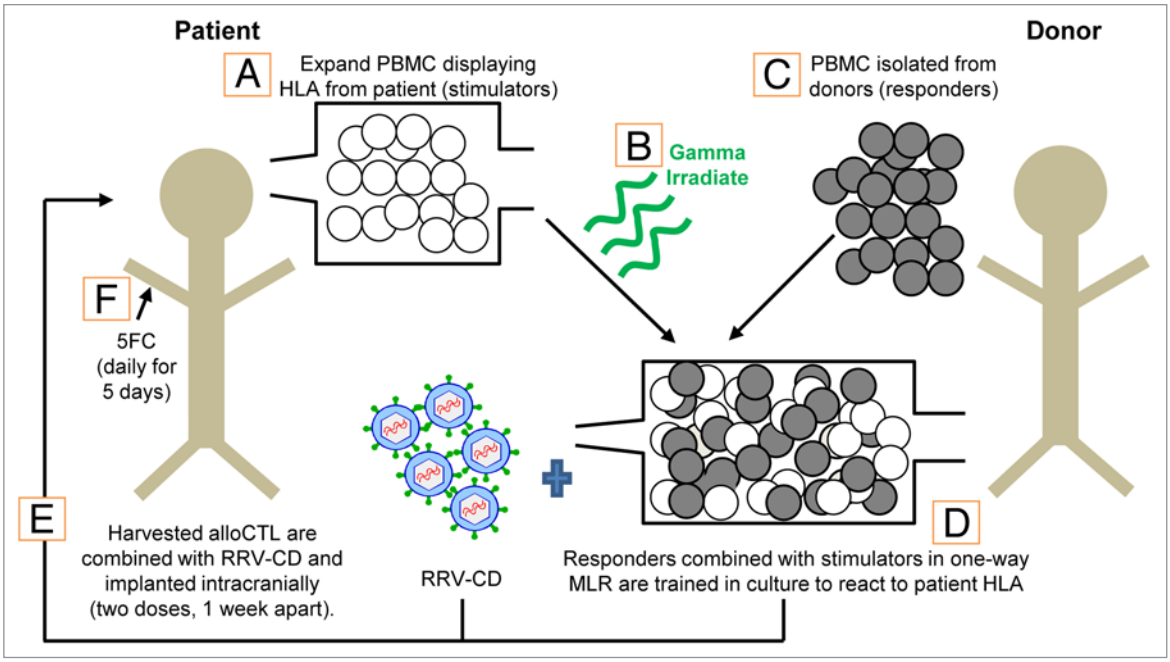

Figure 1. A clinical study design formulated for immuno-gene therapy of brain metastases. The steps show: **(A)** PBMC isolated from patient blood are expanded with high dose Interleukin-2 and OKT3 to serve as stimulators. **(B)** Expanded stimulators are inactivated by irradiation. **(C)** Precursor alloreactive CTL (alloCTL) are derived from healthy allodonor PBMC, are partially HLA-disparate to the patient, and serve as responders. **(D)** Effector alloCTL are generated by one-way mixed lymphocyte reaction (MLR); inactivated stimulators are mixed with responders and cultured with low-dose Interleukin-2. **(E)** Patient undergoes a craniectomy for installation of a reservoir/catheter and to receive an intracranial implant of alloCTL and RRV coding for cytosine deaminase (RRV-CD); one week later more alloCTL + RRV-CD are infused through the catheter. **(F)** Three weeks following the last intracranial infusate the prodrug, 5-flurocytosine (5FC), is administered daily for 5 d to complete a treatment cycle. Multiple treatment cycles are possible. Different HLA-mismatched allodonors are used at each cycle.

and RRV-CD showed a significant survival advantage over single therapeutic modalities (median survival time 97.5 d compared with 50–83 d) and all experimental treatment groups survived significantly longer than sham-treated groups (median survivals 31.5 or 40 d). Vector biodistribution studies within the brain and in extratumoral tissues showed the safety of the approaches, and long-term survivors in gene therapy treatment groups had low or no detectable levels of RRV signals correlating with the apparent absence of tumor by histopathology. Overall, combining the novel alloCTL and RRV approaches provided multiple mechanisms of tumor cell targeted cytotoxicity, including cytotoxic T lymphocyte effector-mediated and chemotherapeutic-mediated cytolysis with suicide vector/prodrug that may be further promoted with bystander effects. The combined therapies are also well tolerated and brain sparing.

Our results demonstrate proof-of-concept that a unique combination regimen consisting of cellular immune and gene therapy approaches is a viable strategy for treatment of established brain metastases. As alloCTL and RRV therapies have now individually reached the clinical testing stage, we can envision the clinical design for combination immunogene therapy of intracranial tumors to be feasible (**Fig. 1**). It is further conceivable that other approaches such as active immunotherapy with dendritic cell vaccination could be added to further augment the immune response to the tumor.[9,10] FDA approval for dendritic cell vaccine therapy for prostate cancer represents an encouraging forward step toward the use of immune therapy as part of a first line regimen. The use of a multi-modal approach will advantageously lead to additive or even synergistic effects elicited by multiple mechanisms of action, i.e., local cytotoxic apoptosis, chemotherapeutic lysis, the generation of an endogenous memory T cell response with proinflammatory cytokine production, and tumor microenvironmental shift from an immunosuppressive to an effector-friendly state. Continued preclinical and clinical investigation of combined, local cellular, gene and vaccine therapy regimens are warranted for primary brain tumors and brain metastases.

Acknowledgments

Financial support for the study was supplied in part by USAMRMC 750 W81XWH-08–1-0734 (CAK), CBCRP 14IB-0114A (BMM), NIH RO1 CA121258 (NK), NIH/NCATS UCLA CTSI Grant Number UL1TR000124 (CAK/NK), the Joan S Holmes Memorial Research Fund (CAK), the Joan S Holmes Memorial Postdoctoral Fellowship (MJH).

Disclosure of Potential Conflicts of Interest

NK has ownership interest (including patents) and is a consultant and advisory board member of Tocagen Inc. The other authors disclose no potential conflicts of interest.

References

1. Nayak L, Lee EQ, Wen PY. Epidemiology of brain metastases. Curr Oncol Rep 2012; 14:48-54; http://dx.doi.org/10.1007/s11912-011-0203-y; PMID:22012633
2. Langley RR, Fidler IJ. The biology of brain metastasis. Clin Chem 2013; 59:180-9; PMID:23115057; http://dx.doi.org/10.1373/clinchem.2012.193342
3. Tabouret E, Chinot O, Metellus P, Tallet A, Viens P, Gonçalves A. Recent trends in epidemiology of brain metastases: an overview. Anticancer Res 2012; 32:4655-62; PMID:23155227
4. Bartsch R, Berghoff AS, Preusser M. Optimal management of brain metastases from breast cancer. Issues and considerations. CNS Drugs 2013; 27:121-34; http://dx.doi.org/10.1007/s40263-012-0024-z; PMID:23239265
5. Hickey MJ, Malone CC, Erickson KE, Gomez GG, Young EL, Liau LM, Prins RM, Kruse CA. Implementing preclinical study findings to protocol design: translational studies with alloreactive CTL for gliomas. Am J Transl Res 2012; 4:114-26; PMID:22347526
6. Ostertag D, Amundson KK, Lopez Espinoza F, Martin B, Buckley T, Galvão da Silva AP, Lin AH, Valenta DT, Perez OD, Ibañez CE, et al. Brain tumor eradication and prolonged survival from intratumoral conversion of 5-fluorocytosine to 5-fluorouracil using a nonlytic retroviral replicating vector. Neuro Oncol 2012; 14:145-59; http://dx.doi.org/10.1093/neuonc/nor199; PMID:22070930
7. Logg CR, Robbins JM, Jolly DJ, Gruber HE, Kasahara N. Retroviral replicating vectors in cancer. Methods Enzymol 2012; 507:199-228; http://dx.doi.org/10.1016/B978-0-12-386509-0.00011-9; PMID:22365776
8. Hickey MJ, Malone CC, Erickson KL, Lin A, Soto H, Ha ET, Kamijima S, Inagaki A, Takahashi M, Kato Y, et al. Combined alloreactive CTL cellular therapy with prodrug activator gene therapy in a model of breast cancer metastatic to the brain. Clin Cancer Res 2013; 19:4137-48; http://dx.doi.org/10.1158/1078-0432.CCR-12-3735; PMID:23780889
9. Prins RM, Wang X, Soto H, Young E, Lisiero DN, Fong B, Everson R, Yong WH, Lai A, Li G, et al. Comparison of glioma-associated antigen peptide-loaded versus autologous tumor lysate-loaded dendritic cell vaccination in malignant glioma patients. J Immunother 2013; 36:152-7; http://dx.doi.org/10.1097/CJI.0b013e3182811ae4; PMID:23377664
10. Qi CJ, Ning YL, Han YS, Min HY, Ye H, Zhu YL, Qian KQ. Autologous dendritic cell vaccine for estrogen receptor (ER)/progestin receptor (PR) double-negative breast cancer. Cancer Immunol Immunother 2012; 61:1415-24; PMID:22290073; http://dx.doi.org/10.1007/s00262-011-1192-2