UTM UNIVERSITI TEKNOLOGI MALAYSIA
RESEARCH UNIVERSITY
International Journal of Innovative Computing
Journal Homepage: http://se.fsksm.utm.my/ijic/index.php/ijic

Synthetic Biology for Breast Cancer Therapy

Wan Fatin Amira Wan Mohd Zawawi and Khairunadwa Jemon[1]
Department of Biosciences and Health Sciences, Faculty of Biosciences and Medical Engineering, Universiti Teknologi Malaysia, 81310 UTM Johor Bahru, Malaysia.
[1]khairun_nadwa@bb.utm.my

***Abstract*— Breast cancer is a heterogenous disease, which can easily metastasize to other part of bodies such as bone and brain, leading to poor prognosis. In relevance to this, targeted therapy with immunotherapy and genetic engineering intervention may represent an ideal approach to destroy the cells from metastasize and prevent tumor recurrence in the future. In this review, we present the current situation of breast cancer therapy in the aspect of synthetic biology as well as its future perspectives. This review highlights the use of engineered cells or organisms as a promising strategy for breast cancer treatment. This includes the use of bacterial therapy, virotherapy and cell-based immunotherapy.**

Keywords — Synthetic biology; breast cancer; immunotherapy

I. INTRODUCTION

Synthetic biology is a multidisciplinary field that makes use of simple organism or living things by manipulating their native genetic makeup and features to improve their efficacy for example in treating disease. The engineered cells will have the ability to overexpress certain desired gene products with enhanced effects, which would be very beneficial to human health and providing better quality of lives. Due to its robust application, there is increasing evidence for the use of synthetic biology approaches for the treatment of various diseases including cardiovascular diseases [1] infectious diseases [2], and cancers [3–5].

Breast cancer is the most common cancer among women worldwide, amounting to 25% of the total cancer incidence [6]. Current treatments for breast cancer rely primarily on surgical resection, chemotherapy and radiotherapy but these treatments have been reported to be associated with some limitations. Although surgical resection is effective on localized tumor but the possibility of recurrence remains as the cancer cells metastasize. Chemotherapy on the other hand was reported to be toxic to normal tissues and thus can destroy normal cells as well [7]. These limitations could be overcome using synthetic biology approaches that allow successful tumor localization and can provide efficacious treatment. In this paper, we first review the construction of modified bacteria and virus to kill the tumors. Further, we discuss the engineering of tools involved in enhancing the immune system including T cells, dendritic cells and antibodies. At the end of this review, we will discuss the future perspectives of growing applications of synthetic biology for breast cancer therapies.

II. BACTERIAL THERAPY

To date, the implementation of synthetic biology in cancer therapies is prominent and seems promising in combating the disease. Bacteria are the perfect candidates for cancer therapy as they equipped with all the important features to destroy cancerous tumors. For instance, they have the ability to specifically target the tumors and produce cytotoxic, anti-cancer proteins [8, 9]. Their cellular circuits and genetic makeup are relatively easy to rewired in order to release the cytotoxic molecules such as lipopolysaccharides [10]. Unlike other biological organisms, what makes bacteria popular in cancer therapies is due to its flagella that can penetrate the tumor tissues [11] and the chemotactic receptors that provide better sensing towards molecular signals in the tumor microenvironment [12, 13]. It was reported that bacterial strain with improper flagella fail to initiate chemotaxis within cylindroids which subsequently preventing them to accumulate and replicate in tumor tissues [12, 13]. This is done by various therapeutic approaches such as direct cell killing, bactofection and as an alternative to gene therapy. However, studies reported that *Salmonella* is the most intriguing bacteria used in breast cancer therapy so far.

A pre-clinical study in murine model has shown that modified auxotrophic strain of *Salmonella typhimurium* was able to cure breast tumors as well as prolonged the survival of the tumor-bearing mice [14]. They also reported that this reisolated leucine-arginine dependent strain known as A1-R strain target breast tumor effectively and the shrinkage of tumor was significantly observed in both *in vivo* and *in vitro* settings. These facultative anaerobic bacteria can grow and multiply in hypoxic microenvironments of tumor as well as in necrotic area indicate that they were able to work alone without any help from chemotherapic drugs [14]. Meanwhile, mutated strain of *S. typhimurium*, VNP200009 has been used

as a vector in delivery of prodrug-activating enzyme carboxypeptidase G2 (CPG2) to breast tumors [1][6]. VNP200009-*S. typhimurium* has also been demonstrated in phase 1 clinical trial to effectively deliver *E. coli* purine nucleoside phosphorylase (ePNP) gene which could convert 6-methylpurine 20-deoxyriboside (MePdR) into a toxic compound, 6-methylpurine (MeP) to solid tumors [16, 17]. Enhanced oncolytic activity in tumors was observed following the activation of pro-drug mediated by these enzyme [15]. Such activity has also been enhanced in *S. typhimurium* by the insertion of gene to express LIGHT cytokine, a TNF-family cytokine that could inhibit primary as well as metastatic growth of breast tumors [18].

Listeria has been extensively studied genus of bacteria that promotes direct cell killing of breast tumors and offers efficient bactofection [16]. Sun *et al.* demonstrated that attenuated *Listeria monocytogenes* (LM)–based vaccine completely eliminates the metastases and induce regression of primary tumor in 4T1 aggressive mouse breast tumor model [19]. This anti tumor activity has been thought to be mediated by two mechanisms; the release of excessive ROS level in tumor microenvironment and the generation of cytotoxic T lymphocytes response against Listeria antigens which these both subsequently promote direct cell lysis and tumor killing [19]. In another report involving *in vivo* breast cancer model, a well-defined attenuated Listeria construct ADXS31-164 was found to be capable in breaking immune tolerance towards the HER2/neu self-antigen, which known to be overexpressed in breast tumors [20]. Meanwhile, the affibody displayed on the surface of engineered *E. coli* BLD21 (DE3) resulted in the internalization of this strain into HER2/neu-positive SKBR-3 breast cancer cells [21]. This non pathogenic tumor-targeting bacteria also carried phage fX174 lysin gene E-mediated autolysis system which promotes autolysis once successfully enter the tumor cells [21]. This demonstrated the use of *E. coli* as an alternative gene therapy.

III. VIROTHERAPY

Besides bacteria, viruses also have widely been used as a synthetic vector for DNA-delivery [22] due to their nature characteristic that can transduce cells efficiently. Several types of viruses including retrovirus, lentivirus, adenovirus, vaccinia virus, and herpes simplex virus [23–25] have been genetically modified to carry the gene of interest and selectively replicate in order to destroy cancer cells. Recombinant viruses like retroviral also involves in the gene-directed enzyme prodrug therapy (GDEPT) that release toxic metabolites from non-toxic pro-drug. For instance, Met-Xia-P450 that carry cythochrome P450 2B6 to activate prodrug cyclophosphamide (CPA) in GDEPT causing regression in growth of MDS-MB-231 breast tumor *in vivo* [26, 27]. Report from clinical trial however revealed discouraging results as only 1% of tumor cells were transduced [28]. Nevertheless, the antitumor activity that was observed warrants further clinical trials. On the other hand, modified retroviral Rexin G equipped with collagen motif has shown its ability to target cancerous lesions [27]. This vector carries a cyclin G1 gene that cause significant reduction of tumor by inducing apoptosis as well as disrupting the proliferation of tumor vasculature [29, 30]. The safety and efficacy of this therapy have been tested in phase I/II study for patients with metastatic breast cancer, in which good prognosis were observed [30]

Virotherapy is typically known to have some limitation for malignant tumor due to the lack specificity of tumor-targeting and inadequate delivery. Interestingly, a decade ago, study by Sarkar *et al.* (2005) has overcome the limitation of insufficient delivery to malignant human breast cancer by dual-specific cancer targeting strategy. This was achieved by manipulating the expression of adenovirus E1a protein which is vital for replication under the control of PEG-3 promoter region together with the introduction of cancer-selective cytotoxic cytokine gene, mda-7/IL-24 [31]. Administration of this adenovirus CRCA (designated Ad.PEG- E1A-mda-7) into mouse model has completely eliminated the primary as well as distant breast tumors [31]. In addition to this, engineered vaccinia virus (VACV) GLV-1h164 has also shown its therapeutic potential in aggressive triple negative breast cancer that is well known to be associated with high level of vascular endothelial growth factor VEGF. Besides the oncolytic activity possessed by the VACV itself, this novel virus encodes for an anti-angiogenic scAb (GLAF-2) and had cause a significant reduction of angiogenesis and tumor sizes [32]. Synergistic oncolytic effect was also observed in GI-101A breast cancer by the combination of a β-galactosidase-activatable prodrug with VACV strain GLV-1h68 [33].

IV. CELL-BASED IMMUNOTHERAPY

Nowadays, cancer immunotherapies are gaining momentum as promising strategy to eradicate tumor cells or at least control the malignancies. The priming of T cell response to target tumor-associated antigen (TAAs) is the fundamental of this therapy. With respect to this matter, T cell itself has widely been engineered to increase its efficacy, and same goes to dendritic cells (DCs) and antibody (Abs) [34–36].

Adoptive T cell therapy involves the use of tumor infiltrating lymphocytes (TILs) particularly T cells that have been removed from cancer patient which will then undergo expansion and infusion back into the patient. In engineered receptor therapies, T cells is extracted form TILs and genetically modified *ex vivo* to express a cancer-targeting receptor and expanded before injected back into patient to treat cancer. There are two types of receptors involves in this approach; T cell receptor (TCR) and chimeric antigen receptor (CAR). These receptors play a significant role in targeting cancer epitopes [37] [38] and markers that expressed on the cell surface, respectively [39]. These receptors have been designed to enhance the specificity and expand the signaling pathways in controlling the therapy.

Genetically modified T cells have been tested in wide range of cancer studies. For instance, complementary CAR-engineered T cells mediated with CD28 that fused to CD3 targeting its ligand; MuC1 and ErbB2 expressed by breast cancer, respectively. Upon binding with its ligand, T- cell proliferation and interleukin (IL)-2 production will be triggered, thus can kill the tumor efficiently [40]. Besides, Herceptin-2 (HER2)-CAR-modified T cells has shown great promise in treating patients with HER2-positive tumors [41]. It is worth to note that phase I/II clinical trial is currently

ongoing to test the safety and efficacy of HER2-CAR-modified T cells for advanced HER2 positive breast cancer (www.clinicaltrials.gov; NCT02547961). Alternatively, studies reported that T cells engineered with protein TARP-specific TCR kills TARP-expressing HLA-$A2^+$ prostate and breast cancer cells effectively, proving that TARP epitope is highly specific and relevant for T-cell therapy [42–44].

Dendritic cells (DCs) are the most potent and powerful antigen presenting cells (APCs) that permits T lymphocytes activation for host immune response inititiation and regulation [45][46]. The manipulation of DCs has emerged as an attractive tool in cancer immunotherapies over the past few decades. The expression of HER-2/*neu* oncogene (ErbB2) has been demonstrated to be overexpressed in breast cancer and hence is an attractive target for immunotherapy. DC-based cancer vaccines in preclinical study by Sakai *et al.* (2004) demonstrated that truncation of *neu* oncogene expressed by DCs prevented the breast tumor growth in BALB-*neu*T transgenic mice [47]. This result to some extent contributes to the development of effective antitumor vaccine strategy. Meanwhile, in phase 1 clinical study, Czerniecki *et al*. (2007) have reported that interferon- and bacterial lipopolysaccharide-activated DC that release high level of IL-12 markedly decreased the HER-2/*neu* expression in breast cancer patients [48]. In addition, APCs (including DC) loaded with sequence of HER-2 extracellular and intracellular domain (designated as HER500) vaccine seems to be well tolerated by patients with breast cancer and this correspond to clinical outcome reported by Peethambaram *et. al* [49]. DC transfected with human tumor antigen MUC1 also increase the mucin-specific interferon-gamma (IFN-γ)-secreting $CD8^+$ T cells in advanced breast cancer patient [50], which implies the feasibility of this vaccination to advanced disease. Favorably, no toxicity and adverse effects recorded in all patients [50].

Recent advancements in antibody engineering have offered the unprecedented expansion of the use of antibodies in cancer immunotherapy. The construction of humanized chimeric antibodies has overcome the limitation of murine antibodies for human use. They have the ability to escape the host immune response and highly specific to cell surface antigens, thus could kill the malignant cells effectively [51][52]. A lot of licensed therapeutic monoclonal antibodies (mAbs) for tumoral diseases have been developed over the last three decades [53]. These mAbs have been used in conjunction with chemotherapeutic regimens as well, in order to create a synergistic effect in killing the cancer cells. For instance, Trastuzumab or commercially known as Herceptin has been developed to particularly target HER-2 receptor and has shown significant clinical benefit in HER-2 positive breast cancer patients [54]. The combination of Trastuzumab with cisplatin, carboplatin, and emtansin have shown positive results and strongly supported the application of this neo-adjuvant chemotherapy [55–57]. Recent idea of engineering multivalent antibodies by Kang *et al.* (2014) to target heregulin-induced HER-3 signaling in breast cancer cells has suggested the use of bispecific anti-HER-2/HER-3 antibody (TAb6) together with lapatinib in breast cancer therapy [58]. This group also reported that tetravalent antibodies are more effective in reducing heregulin-mediated signaling and growth, but bispecific antibody has higher inbitory activity.

On the other hand, ganglioside GD2 is highly expressed on tumor tissue and is restricted in normal tissue except for the brain, thus it has become a suitable target for mAbs in cancer immunotherapy. Moreover, recent studies have identified the presence of GD2 on breast cancer stem cells [59] and led to enhanced proliferation in triple negative breast cancer [60]. The engineered anti-GD2 antibody has been demonstrated in few clinical trials, showing its efficacy in treating neuroblastoma [61], advanced melanoma [62] and lung cancer [63], but however, no clinical trial has been done to prove its efficiency in breast cancer treatment [59]. Nonetheless, the involvement of GD2/Met axis in ER-negative breast cancer aggressiveness firmly suggests the usefulness of anti-GD2 in therapeutic breast cancer treatment [64].

V. FUTURE DIRECTION AND CONCLUDING REMARKS

The implementation of synthetic biology in bacterial therapy, virotherapy and immunotherapies has shown excellent promise in both preclinical and clinical settings. Despite these positive early outcomes, a number of fundamental questions remain to be resolved and further improvements are required before the widespread implementation of these approaches in treating breast cancer.

There is an opportunity for synthetic biology-based approach to be combined with other antitumor therapy. Thermotherapies for instance, are more attractive to both patients and physicians and have shed promising effects in breast cancer treatment. This is because they leave native tissue unaffected and causing less disruption to the contour of the breast hence giving better cosmetic result [65, 66] . What makes this strategy is more interesting is that, in recent study, Unga and Hashida have reported that high-intensity focused ultrasound (HIFU), a type of thermo ablation, can act as immunotherapeutic agent as well due to its ability to activate immune response towards cancer [67]. This reinforces the notion that the combination of thermotherapies and engineered immunotherapeutic agent would possibly be extremely effective in improving current breast cancer therapy. This would create an excellent synergistic effect in killing the cancer, given that adaptive immune response are more potent at elevated temperature [68].

ACKNOWLEDGMENT

We are grateful for the UTM scholarship awarded to Wan Fatin Amira Wan Mohd Zawawi. We would like to thank the Ministry of Higher Education Malaysia (FRGS grant no: 4F729) for funding this research.

REFERENCES

[1] Ye H, Charpin-El Hamri G, Zwicky K, Christen M, Folcher M, Fussenegger M (2013) Pharmaceutically controlled designer circuit for the treatment of the metabolic syndrome. Proc Natl Acad Sci U S A 110:141–6. doi: 10.1073/pnas.1216801110

[2] Ando H, Citorik R, Cleto S, Lemire S, Mimee M, Lu T (2014) Synthetic Biology and Therapies for Infectious Diseases. Nov Antimicrob Agents Strateg 1:109–180. doi: 10.1002/9783527676132.ch7

[3] Anderson JC, Clarke EJ, Arkin AP, Voigt CA (2006) Environmentally controlled invasion of cancer cells by engineered bacteria. J Mol Biol 355:619–627. doi: 10.1016/j.jmb.2005.10.076

[4] Melpignano R (2013) Synthetic Biology for Tumor Therapy.

[5] Collins JJ, Khalil AS (2010) Synthetic biology: applications come of age. Nat Rev Genet 11:367–379. doi: 10.1038/nrg2775

[6] Torre LA, Bray F, Siegel RL, Ferlay J, Lortet-tieulent J, Jemal A (2015) Global Cancer Statistics, 2012. CA a cancer J Clin 65:87–108. doi: 10.3322/caac.21262.

[7] Minchinton AI, Tannock IF (2006) Drug penetration in solid tumors. Nat Rev Cancer 6:583–592. doi: 10.1038/nrc1893

[8] Theys J, Barbé S, Landuyt W, Nuyts S, Van Mellaert L, Wouters B, Anné J, Lambin P (2003) Tumor-specific gene delivery using genetically engineered bacteria. Curr Gene Ther 3:207–21.

[9] Dessel N Van, Swofford CA, Forbes NS (2016) Potent and tumor specific: arming bacteria with therapeutic proteins. 6:385–399. doi: 10.4155/tde.14.113.

[10] Patyar S, Joshi R, Byrav DSP, Prakash a, Medhi B, Das BK (2010) Bacteria in cancer therapy: a novel experimental strategy. J Biomed Sci 17:21. doi: 10.1186/1423-0127-17-21

[11] Forbes NS (2010) Engineering the perfect (bacterial) cancer therapy. Nat Rev Cancer 10:785–794. doi: 10.1038/nrc2934

[12] Kasinskas RW, Forbes NS (2007) Salmonella typhimurium lacking ribose chemoreceptors localize in tumor quiescence and induce apoptosis. Cancer Res 67:3201–3209. doi: 10.1158/0008-5472.CAN-06-2618

[13] Kreuter J (1996) Nanoparticles and microparticles for drug and vaccine delivery. J Anat 189 (Pt 3:503–505. doi: 10.1002/bit

[14] Zhao M, Yang M, Ma H, Li X, Tan X, Li S, Yang Z, Hoffman RM (2006) Targeted therapy with a Salmonella typhimurium leucine-arginine auxotroph cures orthotopic human breast tumors in nude mice. Cancer Res 66:7647–7652. doi: 10.1158/0008-5472.CAN-06-0716

[15] Friedlos F, Lehouritis P, Ogilvie L, Hedley D, Davies L, Bermudes D, King I, Martin J, Marais R, Springer CJ (2008) Attenuated Salmonella targets prodrug activating enzyme carboxypeptidase G2 to mouse melanoma and human breast and colon carcinomas for effective suicide gene therapy. Clin Cancer Res 14:4259–4266. doi: 10.1158/1078-0432.CCR-07-4800

[16] Bunting A (2013) Genetically engineered bacteria as cancer fighting agents. Univ Ottawa Med J 3:26–33.

[17] Fu W, Lan H, Li S, Han X, Gao T, Ren D (2008) Synergistic antitumor efficacy of suicide/ePNP gene and 6-methylpurine 2'-deoxyriboside via Salmonella against murine tumors. Cancer Gene Ther 15:474–484. doi: 10.1038/cgt.2008.19

[18] Loeffler M, Le'Negrate G, Krajewska M, Reed JC (2007) Attenuated Salmonella engineered to produce human cytokine LIGHT inhibit tumor growth. Proc Natl Acad Sci U S A 104:12879–12883. doi: 10.1073/pnas.0701959104

[19] Sun HK, Castro F, Paterson Y, Gravekamp C (2009) High efficacy of a Listeria-based vaccine against metastatic breast cancer reveals a dual mode of action. Cancer Res 69:5860–5866. doi: 10.1158/0008-5472.CAN-08-4855

[20] Shahabi V, Seavey MM, Maciag PC, Rivera S, Wallecha a (2011) Development of a live and highly attenuated Listeria monocytogenes-based vaccine for the treatment of Her2/neu-overexpressing cancers in human. Cancer Gene Ther 18:53–62. doi: 10.1038/cgt.2010.48

[21] Chang CH, Cheng WJ, Chen SY, Kao MC, Chiang CJ, Chao YP (2011) Engineering of Escherichia coli for targeted delivery of transgenes to HER2/neu-positive tumor cells. Biotechnol Bioeng 108:1662–1672. doi: 10.1002/bit.23095

[22] Roth CM, Sundaram S (2004) Engineering synthetic vectors for improved DNA delivery: insights from intracellular pathways. Annu Rev Biomed Eng 6:397–426. doi: 10.1146/annurev.bioeng.6.040803.140203

[23] Takahashi S, Ito Y, Hatake K, Sugimoto Y (2006) Gene Therapy for Breast Cancer . – Review of Clinical Gene Therapy Trials for Breast Cancer and MDR1 Gene Therapy Trial in Cancer Gene Therapy for Breast Cancer. Cancer 13:

[24] Gholami S, Chen C-H, Belin LJ, Lou E, Fujisawa S, Antonacci C, Carew A, Chen NG, De Brot M, Zanzonico PB, Szalay A a, Fong Y (2013) Vaccinia virus GLV-1h153 is a novel agent for detection and effective local control of positive surgical margins for breast cancer. Breast Cancer Res 15:R26. doi: 10.1186/bcr3404

[25] Takakuwa H, Goshima F, Nozawa N, Yoshikawa T, Kimata H, Nakao A, Nawa A, Kurata T, Sata T, Nishiyama Y (2003) Oncolytic viral therapy using a spontaneously generated herpes simplex virus type 1 variant for disseminated peritoneal tumor in immunocompetent mice. Arch Virol 148:813–825. doi: 10.1007/s00705-002-0944-x

[26] Kan O, Griffiths L, Baban D, Iqball S, Uden M, Spearman H, Slingsby J, Price T, Esapa M, Kingsman S, Kingsman a, Slade a, Naylor S (2001) Direct retroviral delivery of human cytochrome P450 2B6 for gene-directed enzyme prodrug therapy of cancer. Cancer Gene Ther 8:473–482. doi: 10.1038/sj.cgt.7700329

[27] McCrudden CM, McCarthy HO (2014) Current status of gene therapy for breast cancer: progress and challenges. Appl Clin Genet 7:209–20. doi: 10.2147/TACG.S54992

[28] Braybrooke JP, Slade A, Deplanque G, Harrop R, Madhusudan S, Forster MD, Gibson R, Makris A, Talbot DC, Steiner J, White L, Kan O, Naylor S, Carroll MW, Kingsman SM, Harris AL (2005) Phase I study of MetXia-P450 gene therapy and oral cyclophosphamide for patients with advanced breast cancer or melanoma. Clin Cancer Res 11:1512–20. doi: 10.1158/1078-0432.CCR-04-0155

[29] Gordon EM, Levy JP, Reed RA, Petchpud WN, Liu L, Wendler CB, Hall FL (2008) Targeting metastatic cancer from the inside: A new generation of targeted gene delivery vectors enables personalized cancer vaccination in situ. Int J Oncol 33:665–675. doi: 10.3892/ijo_00000052

[30] Gordon EM, Hall FL (2010) Noteworthy clinical case studies in cancer gene therapy: Tumortargeted rexin-G advances as an efficacious anti-cancer agent. Int J Oncol 36:1341–1353. doi: 10.3892/ijo-00000619

[31] Sarkar D, Su ZZ, Vozhiila N, Park ES, Gupta P, Fisher PB (2005) Dual cancer-specific targeting strategy cures primary and distant breast carcinomas in nude mice. Proc Natl Acad Sci U S A 102:14034–14039. doi: Doi 10.1073/Pnas.0506837102

[32] Gholami S, Marano A, Chen NG, Aguilar RJ, Frentzen A, Chen CH, Lou E, Fujisawa S, Eveno C, Belin L, Zanzonico P, Szalay A, Fong Y (2014) A novel vaccinia virus with dual oncolytic and anti-angiogenic therapeutic effects against triple-negative breast cancer. Breast Cancer Res Treat 148:489–499. doi: 10.1007/s10549-014-3180-7

[33] Seubert CM, Stritzker J, Hess M, Donat U, Sturm JB, Chen N, von Hof JM, Krewer B, Tietze LF, Gentschev I, Szalay AA (2011) Enhanced tumor therapy using vaccinia virus strain GLV-1h68 in combination with a beta-galactosidase-activatable prodrug seco-analog of duocarmycin SA. Cancer Gene Ther

18:42–52. doi: 10.1038/cgt.2010.49
[34] Sadelain M (2009) T-cell engineering for cancer immunotherapy. Cancer J 15:451–455. doi: 10.1097/PPO.0b013e3181c51f37
[35] Boudreau JE, Bonehill A, Thielemans K, Wan Y (2011) Engineering dendritic cells to enhance cancer immunotherapy. Mol Ther 19:841–53. doi: 10.1038/mt.2011.57
[36] Kim SJ, Park Y, Hong HJ (2005) Antibody engineering for the development of therapeutic antibodies. Mol Cells 20:17–29.
[37] Thaxton JE, Li Z (2015) To affinity and beyond: Harnessing the T Cell receptor for cancer immunotherapy. 37–41. doi: 10.4161/21645515.2014.973314
[38] Johnson LA, Morgan RA, Dudley ME, Cassard L, Yang JC, Hughes MS, Kammula US, Royal RE, Sherry RM, Wunderlich JR, Lee CCR, Restifo NP, Schwarz SL, Cogdill AP, Bishop RJ, Kim H, Brewer CC, Rudy SF, VanWaes C, Davis JL, Mathur A, Ripley RT, Nathan DA, Laurencot CM, Rosenberg SA (2009) Gene therapy with human and mouse T-cell receptors mediates cancer regression and targets normal tissues expressing cognate antigen. Blood 114:535–546. doi: 10.1182/blood-2009-03-211714
[39] Chakravarti D, Wong WW (2015) Synthetic biology in cell-based cancer immunotherapy. Trends Biotechnol 33:449–461. doi: 10.1016/j.tibtech.2015.05.001
[40] Wilkie S, Van Schalkwyk MCI, Hobbs S, Davies DM, Van Der Stegen SJC, Pereira ACP, Burbridge SE, Box C, Eccles SA, Maher J (2012) Dual targeting of ErbB2 and MUC1 in breast cancer using chimeric antigen receptors engineered to provide complementary signaling. J Clin Immunol 32:1059–1070. doi: 10.1007/s10875-012-9689-9
[41] Ahmed N, Brawley VS, Hegde M, Robertson C, Ghazi A, Gerken C, Liu E, Dakhova O, Ashoori A, Corder A, Gray T, Wu M-F, Liu H, Hicks J, Rainusso N, Dotti G, Mei Z, Grilley B, Gee A, Rooney CM, Brenner MK, Heslop HE, Wels WS, Wang LL, Anderson P, Gottschalk S (2015) Human Epidermal Growth Factor Receptor 2 (HER2) -Specific Chimeric Antigen Receptor-Modified T Cells for the Immunotherapy of HER2-Positive Sarcoma. J Clin Oncol 33:1688–96. doi: 10.1200/JCO.2014.58.0225
[42] Carlsson B, Tötterman TH, Essand M (2004) Generation of cytotoxic T lymphocytes specific for the prostate and breast tissue antigen TARP. Prostate 61:161–170. doi: 10.1002/pros.20091
[43] Epel M, Carmi I, Soueid-Baumgarten S, Oh SK, Bera T, Pastan I, Berzofsky J, Reiter Y (2008) Targeting TARP, a novel breast and prostate tumor-associated antigen, with T-cell receptor- like human recombinant antibodies. Eur J Immunol 38:1706–1720. doi: 10.1002/eji.200737524.Targeting
[44] Hillerdal V, Nilsson B, Carlsson B, Eriksson F, Essand M (2012) T cells engineered with a T cell receptor against the prostate antigen TARP specifically kill HLA-A2+ prostate and breast cancer cells. Proc Natl Acad Sci 109:15877–15881. doi: 10.1073/pnas.1209042109
[45] Bhargava A, Mishra D, Banerjee S, Mishra PK (2012) Dendritic cell engineering for tumor immunotherapy: from biology to clinical translation. Immunotherapy 4:703–718. doi: 10.2217/imt.12.40
[46] Banchereau J, Steinman RM (1998) Dendritic cells and the control of immunity. Nature 392:245–252. doi: 10.1038/32588
[47] Sakai Y, Morrison BJ, Burke JD, Park J, Terabe M, Janik JE, Forni G, Berzofsky JA, Morris JC (2004) Vaccination by Genetically Modified Dendritic Cells Expressing a Truncated neu Oncogene Prevents Development of Breast Cancer in Transgenic Mice Vaccination by Genetically Modified Dendritic Cells Expressing a Truncated neu Oncogene Prevents Development o. Cancer Res 8022–8028. doi: 10.1158/0008-5472.CAN-03-3442
[48] Czerniecki BJ, Koski GK, Koldovsky U, Xu S, Cohen PA, Mick R, Nisenbaum H, Pasha T, Xu M, Fox KR, Weinstein S, Orel SG, Vonderheide R, Coukos G, DeMichele A, Araujo L, Spitz FR, Rosen M, Levine BL, June C, Zhang PJ (2007) Targeting HER-2/neu in early breast cancer development using dendritic cells with staged interleukin-12 burst secretion. Cancer Res 67:1842–1852. doi: 10.1158/0008-5472.CAN-06-4038
[49] Peethambaram PP, Melisko ME, Rinn KJ, Alberts SR, Provost NM, Jones LA, Sims RB, Lin LRC, Frohlich MW, Park JW (2009) A phase I trial of immunotherapy with lapuleucel-T (APC8024) in patients with refractory metastatic tumors that express HER-2/neu. Clin Cancer Res 15:5937–5944. doi: 10.1158/1078-0432.CCR-08-3282
[50] Pecher G, Häring A, Kaiser L, Thiel E (2002) Mucin gene (MUC1) transfected dendritic cells as vaccine: Results of a phase I/II clinical trial. Cancer Immunol Immunother 51:669–673. doi: 10.1007/s00262-002-0317-z
[51] Mohindru M, Verma A (2005) Engineered antibodies act as targeted therapies in cancer treatment. Indian J Pediatr 72:943–947.
[52] Ahmed M, Cheung NK V (2014) Engineering anti-GD2 monoclonal antibodies for cancer immunotherapy. FEBS Lett 588:288–297. doi: 10.1016/j.febslet.2013.11.030
[53] Buss NAPS, Henderson SJ, McFarlane M, Shenton JM, De Haan L (2012) Monoclonal antibody therapeutics: History and future. Curr Opin Pharmacol 12:615–622. doi: 10.1016/j.coph.2012.08.001
[54] Gajria D, Chandarlapaty S (2011) HER2-amplified breast cancer: mechanisms of trastuzumab resistance and novel targeted therapies. Expert Rev Anticancer Ther 11:263–75. doi: 10.1586/era.10.226
[55] Huang R, Wang Q, Zhang X, Zhu J, Sun B (2015) Trastuzumab-cisplatin conjugates for targeted delivery of cisplatin to HER2-overexpressing cancer cells. Biomed Pharmacother 72:17–23. doi: 10.1016/j.biopha.2015.03.004
[56] Kolberg H-C, Akpolat-Basci L, Stephanou M, Hannig C, Liedtke C (2015) Neoadjuvant chemotherapy with docetaxel, carboplatin, and weekly trastuzumab (TCH) activity in HER2-positive early breast cancer: Results after a median follow-up of 4.5 years. Abstract 140. J Clin Oncol 33:140.
[57] Verma S, Miles D, Gianni L, Krop IE, Welslau M, Baselga J, Pegram M, Oh D-Y, Diéras V, Guardino E, Fang L, Lu MW, Olsen S, Blackwell K (2012) Trastuzumab emtansine for HER2-positive advanced breast cancer. N Engl J Med 367:1783–91. doi: 10.1056/NEJMoa1209124
[58] Kang JC, Poovassery JS, Bansal P, You S, Manjarres IM, Ober RJ, Ward ES (2014) Engineering multivalent antibodies to target heregulin-induced HER3 signaling in breast cancer cells. MAbs 6:340–353. doi: 10.4161/mabs.27658
[59] Battula VL, Shi Y, Evans KW, Wang RY, Spaeth EL, Jacamo RO, Guerra R, Sahin AA, Marini FC, Hortobagyi G, Mani SA, Andreeff M (2012) Ganglioside GD2 identifies breast cancer stem cells and promotes tumorigenesis. J Clin Invest 122:2066–2078. doi: 10.1172/JCI59735
[60] Cazet A, Bobowski M, Rombouts Y, Lefebvre J, Steenackers A, Popa I, Guérardel Y, Le Bourhis X, Tulasne D, Delannoy P (2012) The ganglioside GD2 induces the constitutive activation of c-Met in MDA-MB-231 breast cancer cells expressing the GD3 synthase. Glycobiology 22:806–816. doi: 10.1093/glycob/cws049
[61] Modak S, Cheung N-K V (2007) Disialoganglioside directed

immunotherapy of neuroblastoma. Cancer Invest 25:67–77. doi: 10.1080/07357900601130763

[62] Osorio M, Gracia E, Reigosa E, Hernandez J, de la Torre A, Saurez G, Perez K, Viada C, Cepeda M, Carr A, Avila Y, Rodríguez M, Fernandez LE (2012) Effect of vaccination with N-glycolyl GM3/VSSP vaccine by subcutaneous injection in patients with advanced cutaneous melanoma. Cancer Manag Res 4:341–5. doi: 10.2147/CMAR.S22617

[63] Alfonso S, Valdés-Zayas A, Santiesteban ER, Flores YI, Areces F, Hernández M, Viada CE, Mendoza IC, Guerra PP, García E, Ortiz RA, de la Torre A V, Cepeda M, Pérez K, Chong E, Hernández AM, Toledo D, González Z, Mazorra Z, Crombet T, Pérez R, Vázquez AM, Macías AE (2014) A randomized, multicenter, placebo-controlled clinical trial of racotumomab-alum vaccine as switch maintenance therapy in advanced non-small cell lung cancer patients. Clin Cancer Res 20:3660–71. doi: 10.1158/1078-0432.CCR-13-1674

[64] Gu Y, Julien S, Delannoy P (2015) Gangliosides in Breast Cancer : New Perspectives. 80:808–819.

[65] Wu F, Wang ZB, Cao YD, Zhu XQ, Zhu H, Chen WZ, Zou JZ (2007) “Wide local ablation” of localized breast cancer using high intensity focused ultrasound. J Surg Oncol 96:130–136. doi: 10.1002/jso.20769

[66] Zhao Z, Wu F (2010) Minimally-invasive thermal ablation of early-stage breast cancer: a systemic review. Eur J Surg Oncol 36:1149–1155. doi: 10.1016/j.ejso.2010.09.012

[67] Unga J, Hashida M (2014) Ultrasound induced cancer immunotherapy ☆. Adv Drug Deliv Rev 72:144–153. doi: 10.1016/j.addr.2014.03.004

[68] Evans SS, Repasky E a, Fisher DT (2015) Fever and the thermal regulation of immunity: the immune system feels the heat. Nat Rev Immunol 15:335–349. doi: 10.1038/nri3843