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PATHOBIOLOGY IN FOCUS

T-lymphocyte homing: an underappreciated yet critical hurdle for successful cancer immunotherapy

Robert Sackstein[1,2,3,4], Tobias Schatton[1,3,5,6] and Steven R Barthel[1,3,5]

Advances in cancer immunotherapy have offered new hope for patients with metastatic disease. This unfolding success story has been exemplified by a growing arsenal of novel immunotherapeutics, including blocking antibodies targeting immune checkpoint pathways, cancer vaccines, and adoptive cell therapy (ACT). Nonetheless, clinical benefit remains highly variable and patient-specific, in part, because all immunotherapeutic regimens vitally hinge on the capacity of endogenous and/or adoptively transferred T-effector (T_{eff}) cells, including chimeric antigen receptor (CAR) T cells, to home efficiently into tumor target tissue. Thus, defects intrinsic to the multi-step T-cell homing cascade have become an obvious, though significantly underappreciated contributor to immunotherapy resistance. Conspicuous have been low intralesional frequencies of tumor-infiltrating T-lymphocytes (TILs) below clinically beneficial threshold levels, and peripheral rather than deep lesional TIL infiltration. Therefore, a T_{eff} cell 'homing deficit' may arguably represent a dominant factor responsible for ineffective immunotherapeutic outcomes, as tumors resistant to immune-targeted killing thrive in such permissive, immune-vacuous microenvironments. Fortunately, emerging data is shedding light into the diverse mechanisms of immune escape by which tumors restrict T_{eff} cell trafficking and lesional penetrance. In this review, we scrutinize evolving knowledge on the molecular determinants of T_{eff} cell navigation into tumors. By integrating recently described, though sporadic information of pivotal adhesive and chemokine homing signatures within the tumor microenvironment with better established paradigms of T-cell trafficking under homeostatic or infectious disease scenarios, we seek to refine currently incomplete models of T_{eff} cell entry into tumor tissue. We further summarize how cancers thwart homing to escape immune-mediated destruction and raise awareness of the potential impact of immune checkpoint blockers on T_{eff} cell homing. Finally, we speculate on innovative therapeutic opportunities for augmenting T_{eff} cell homing capabilities to improve immunotherapy-based tumor eradication in cancer patients, with special focus on malignant melanoma.

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Cancer treatment has entered a revolutionary era with the dawn of innovative therapies capable of harnessing the immune system to destroy tumors. These novel immune-boosting approaches, collectively known as 'immunotherapeutics', are currently in the vanguard of personalized, precision-guided medicine, and offer unprecedented hope to patients with advanced, metastatic cancer. Compartmentalized into three distinct treatment modes, cancer immunotherapies now include: (1) Vaccines for immunizing against tumor antigens (TAs); (2) adoptive cell therapy (ACT) wherein *ex vivo* expanded immune effector cells are infused into patients; and (3) immunomodulators for improving patient-intrinsic anti-cancer immunity.[1–3] Vital to the clinical success of all three regimens in eradicating or restraining cancer progression is the logistical dependency for efficient homing and entry of effector immunocytes, especially T cells, into the heart of primary and metastatic lesional tissue.

The term tumor-infiltrating lymphocyte (TIL) was originally coined by Wallace Clark in 1969 and later defined operationally as a lymphocyte that has left the bloodstream and has gained direct contact with tumor cells. More recently, the term TIL has been used to describe a variety of tumor-infiltrating cells including T cells, T regulatory (T_{reg}) cells,

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[1]Department of Dermatology, Brigham and Women's Hospital, Harvard Medical School, Boston, MA, USA; [2]Department of Medicine, Brigham and Women's Hospital, Harvard Medical School, Boston, MA, USA; [3]Harvard Skin Disease Research Center, Brigham and Women's Hospital, Harvard Medical School, Boston, MA, USA; [4]Program of Excellence in Glycosciences, Harvard Medical School, Boston, MA, USA; [5]Harvard Stem Cell Institute, Harvard Medical School, Boston, MA, USA and [6]Department of Medicine, Boston Children's Hospital, Harvard Medical School, Boston, MA, USA

Correspondence: Steven R Barthel, PhD, Department of Dermatology, Brigham and Women's Hospital, Harvard Medical School, Room 673B, 77 Avenue Louis Pasteur, Boston, MA 02115, USA.
E-mail: sbarthel@research.bwh.harvard.edu

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natural killer (NK) cells, and B cells, as well as macrophages, dendritic cells (DC), and myeloid-derived suppressor cells (MDSC).[4] Herein we use the term 'TIL' in reference selectively to the lymphocytotoxic arm of tumor immunity comprised of cytotoxic $CD8^+$ T-effector (T_{eff}) cells given their robust tumoricidal and peripheral tissue-homing capacity, characteristics not typically found in related $CD8^+$ central memory T-cell subsets (T_{cm}).[5–8] This emphasis on T_{eff} cells also does not overlook the fact that all TILs, including NK cells, have participatory roles at the tumor-immune synapse in cancer immunoreactivity and by extension in enhancing or blunting responses to immunotherapy, but underscores the fact that the final most prominent and comprehensively analyzed anti-tumor attack is exerted by cytotoxic lymphocytes (primarily $CD8^+$ T_{eff} cells) and supported by NK cells as well as $CD4^+$ T cells of Th1 (IFN-γ)-producing phenotype.[9] These assailants must employ an ensemble of homing molecules enabling navigation into and subsequent destruction of neoplastic targets. We further discuss how the current efforts at creation and culture-expansion of adoptively transferred T_{eff} cells, defined herein as ACT_{eff} cells, and which have further applicability to NK cells, must include strategies to optimize delivery of these cells to sites where they are needed. To further simplify and where appropriate, we use the term T_{eff} to describe T cells of both endogenous (TIL) and exogenously expanded (ACT_{eff}) sources.

There are a variety of recent melanoma and solid cancer clinical trials wherein monoclonal antibody (mAb) blockade of immune checkpoint receptor pathways, including programmed cell death protein-1 (PD-1; pembrolizumab, nivolumab) and its ligand programmed death-ligand 1 (PD-L1; MPDL3280A), and cytotoxic T-lymphocyte-associated protein-4 (CTLA-4; ipilimumab), have shown exciting potential in reversing T_{eff} cell dysfunction and exhaustion thereby enhancing their attack on and shrinkage of late-stage metastases in patients for which little or no hope was previously available.[10–12] Despite such advances, several challenges exist with use of immune checkpoint agents, including variable response rates in less than half of patients with advanced melanoma (and with even lower efficacy against other cancers deemed 'less immunogenic'), potential effects on newly discovered immune checkpoint pathways intrinsic to tumor cells, and potential effects on T_{eff} cell homing.[11,13] Importantly, emerging data now implicates defects in T_{eff} cell homing as a critical factor in resistance to immune checkpoint blockade. In support, while circulating T-cell numbers and activation status in peripheral blood alone do not routinely coincide with either anti-tumor activity, prognosis, or survival as originally thought, TIL frequency, density, spatial localization, and subset ratio intrinsically within tissue of melanoma and other solid tumors correlates well with favorable prognosis and immunotherapeutic responses.[4,14] Indeed, the ratio of intralesional $CD8^+$ T cells to either T_{reg} or $CD4^+$ T cells has been construed as a superior predictive criterion of patient outcome than conventional tumor node-metastasis (TNM) staging.[9,15] Thus, immunotherapeutic success critically hinges upon efficient homing of TIL or ACT_{eff} cell subsets from the circulation into the inflamed tumor compartment.

Optimization of TIL and ACT_{eff} cell trafficking schemas depends on a thorough understanding of the dynamic T_{eff} cell homing circuitry, its repertoire of highly integrated components, inherent defects, and diverse modes by which tumors hijack such processes. The T_{eff} cell 'homing deficit' is a formidable hurdle as tumors have evolved multiple, diverse immunoevasive tricks to thwart immunocyte lesional penetrance, among which include downregulation or masking of TAs along with tumor-induced aberrancies in the expression of adhesive, chemokine, and other pro-migratory molecules intrinsic either to immunocytes themselves or to accessory partners in their homing cascade, eg, tumor microvessels, tumor cells, or stroma. Inasmuch, new treatments aimed at replenishing recruitment factors to render tumors permissive to T_{eff} cell infiltration and attack might enhance ACT and/or synergize with clinically-approved immune checkpoint mAbs and other regimens to greatly reduce variability and augment efficacy of T_{eff} cell-directed immunotherapy approaches.

Unfortunately, identity and function of tumor-targeting T_{eff} cell homing mediators have been gleaned from only a sparse cohort of studies interrogating the TIL or ACT_{eff} cell migratory apparatus directly as reviewed subsequently in this article. To compensate for the paucity of homing-related data, we overlay the substantive historical knowledge of T-cell trafficking as it occurs under steady-state, homeostatic, or infectious scenarios (Part I) onto the spottier recent data on T_{eff} cell homing processes into malignant tissue (Part II) and seek to refine understanding of how T_{eff} cells infiltrate tumors, how cancers thwart such migration to avoid immune-targeted killing, and raise awareness of the possible unexpected impact of immune checkpoint blockers on T_{eff} cell homing. We then integrate this information in describing new translational options for better steering T_{eff} cells, eg, TIL and ACT_{eff}, into direct confrontation with tumor tissue (Part III) and offer our concluding opinions for improving immunotherapeutic outcomes for cancer patients.

THE CONVENTIONAL MULTI-STEP PARADIGM OF T-CELL HOMING

Immune resistance to infection and cancer is controlled spatiotemporally by a coordinated arrangement of rolling and adhesive steps enabling circulating leukocytes, and importantly T cells, to extravasate and infiltrate diseased tissue under hemodynamic flow conditions. Vital to the success of this extravasation cascade, and by extension to the immunotherapeutic control of cancer, is the acquisition of highly specialized T-cell 'homing' receptors, which metaphorically resemble postal addresses and zip codes in their enablement of T-cell organotropic targeting in response to conversion from naive to antigen-experienced cells (Table 1; Figures 1 and 2). The steps in this cascade involve: (1) tethering and

Table 1 T_{eff} cell homing receptors and their cognate ligands mediating organotropic targeting

Homing tissue type	T_{eff} cell homing receptor	Cognate ligand
Skin	CLA (PSGL-1 glycoform)	E/P-selectin
	CD43E	E-selectin
	VLA-4 ($\alpha_4\beta_1$)	VCAM-1
	LFA-1 ($\alpha_L\beta_2$)	ICAM-1
	CCR4	CCL17
	CCR10	CCL27
Gut (intestine, colon, mLN, PP)	$\alpha_4\beta_7$	MAdCAM-1
	CCR9[a]	CCL25[a]
	CXCR4	CXCL12
	Selectin ligands[b]	E/P-selectin[b]
	VLA-4[b]	VCAM-1[b]
	LFA-1[b]	ICAM-1[b]
	CCR6[b]	CCL20 (MIP-3α)[b]
Liver	CD44	Hyaluronate
	VLA-4	VCAM-1
	CCR5	CCL5
	?	VAP-1
	Selectin ligands[b]	E/P-selectin
	$\alpha_4\beta_7$[b]	MAdCAM-1[b]
Lung	LFA-1	ICAM-1
	CCR3	CCL28
	CCR4	CCL17
	CXCR4	CXCL12
	Selectin ligands[b]	E/P-selectin[b]
	VLA-4[b]	VCAM-1[b]
	LFA-1[b]	ICAM-1[b]
Bone marrow	CLA (PSGL-1 glycoform)	E/P-selectin
	CD43E	E-selectin
	VLA-4	VCAM-1
	LFA-1	ICAM-1
	CXCR4	CXCL12
	$\alpha_4\beta_7$[b]	MAdCAM-1[b]
Heart	CCR5	CCL4, CCL5
	CCR4	?
	CXCR3	CXCL10
	c-Met	HGF
Brain	VLA-4[b]	VCAM-1[b]
	LFA-1[b]	ICAM-1[b]
	CXCR3[b]	CXCL9/CXCL10[b]
Peripheral LN[c]	Selectin ligands[b]	E/P-selectin[b]
	LFA-1[b]	ICAM-1[b]
	CXCR3[b]	CXCL9/CXCL10[b]

[a] Involved in T_{eff} cell homing to the intestine but not colon.
[b] Inflammatory reactions, tissue injury.
[c] Under non-inflamed, steady-state conditions, T_{eff} cells typically lose L-selectin and CCR7 expression and are largely restricted from LN access though may enter during inflammatory reactions (b) as shown. In contrast, both naive T cells and T_{cm} cells express L-selectin, CCR7, and CXCR4 and engage PNAd, CCL19/CCL21, and CXCL12, respectively, to undergo T-cell rolling and LFA-1/ICAM-1/2- mediated adhesion and transmigration into LNs.

rolling adhesive interactions of the blood-borne cell onto the endothelial surface (ie, deceleration against the prevailing forces of blood flow); (2) integration of chemokine-mediated signaling within the milieu (via chemokine receptors expressed on the circulating cell), leading to integrin activation; (3) integrin-mediated firm adherence of the cell onto the endothelial surface; and (4) endothelial transmigration. As T cells exploit identical homing molecules for stepwise extravasation into diverse normal tissues as well as into tumors, a greater understanding of the native T-cell trafficking machinery and its roadmap will undoubtedly benefit immunotherapeutic strategies to enhance TIL and ACT_{eff} cell infiltration of tumors.

Steady-State Homing and Recirculation of Naive T Cells into Lymphoid Tissues

Naive T cells, first born and maturing in primary lymphoid organs of the bone marrow and thymus, respectively, recirculate under steady-state homeostatic conditions, carried by a network of liquid conduits of blood and lymphatic vessels to a diverse ensemble of dispersed secondary lymphoid organs (SLO), including hundreds of lymph nodes (LNs).[29] Arrest on specialized LN postcapillary venules (known as high endothelial venules (HEV)) requires T cells to apply adhesive 'brakes' acting like velcro to resist the momentum of hemodynamic flow. These initial tethering and rolling HEV contacts are principally mediated by glycan-dependent receptor/ligand interactions, prompted by leukocyte (L)-selectin (CD62L) on naive T cells engaging with pertinent ligands on HEV which are collectively termed 'peripheral LN addressins' (PNAd), and consist of a family of sialylated mucins (sialomucins) that include the glycoproteins CD34, podocalyxin, endomucin, nepmucin (CLM9), and glycosylation-dependent cell adhesion molecule 1 (GLYCAM1; found only in mice), and in some cases, L-selectin may also bind endothelial-expressed P-selectin glycoprotein ligand 1 (PSGL-1).[29–31] The selectins are a family of three lectins consisting of L-selectin (CD62L, expressed on leukocytes and hematopoietic stem/progenitor cells), and the 'vascular selectins' E-selectin (CD62E, expressed on endothelial cells) and P-selectin (CD62P, expressed on endothelial cells and platelets). All three selectins bind in a Ca^{2+}-dependent fashion to a sialofucosylated tetrasaccharide motif known as 'sialylated Lewis X' (sLe^X, also known as CD15s: NeuAcα(2-3)Galβ(1-4)[Fucα(1-3)]GlcNAcβ(1-R)). PNAd molecules contain a sulfated form of this tetrasaccharide and are synthesized in part by α(1,3)-fucosyltransferases (FT)-IV and -VII and N-acetylglucosamine 6-*O*-sulphotransferase.[29] Next, CC-chemokine receptor 7 (CCR7) expressed on rolling, naive T cells binds chemokines CCL19 and CCL21, and, in combination with minor engagement of CXC-chemokine receptor 4 (CXCR4) with CXCL12 (stromal cell-derived factor 1, SDF1), elicits a signaling cascade and rapid downstream activation of the T-cell β_2-integrin LFA-1 ($\alpha_L\beta_2$).[5–7,30] Chemokine-induced activation of LFA-1 is further enhanced by HEV-expressed glycosaminoglycans (GAGs) such as heparin sulfate, which

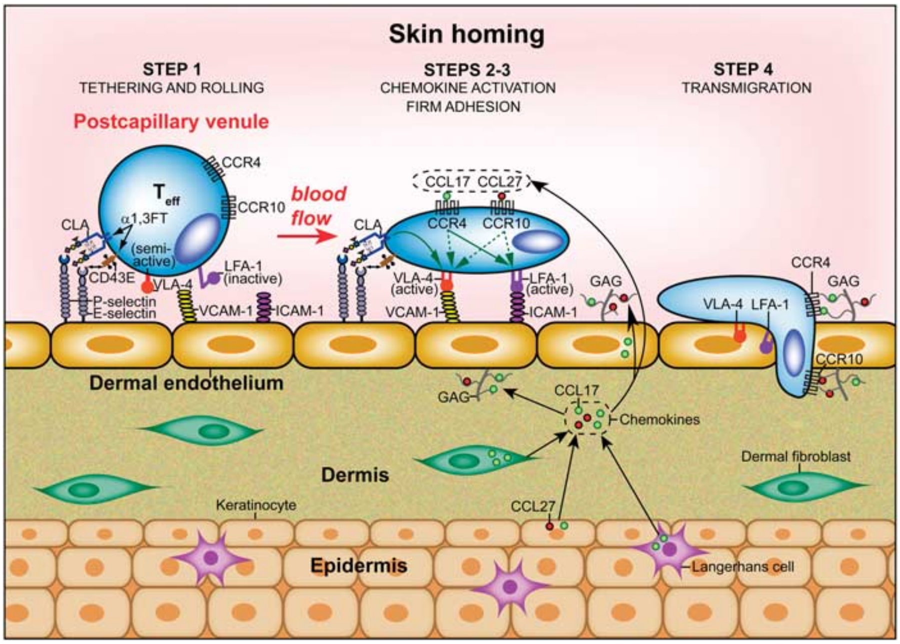

Figure 1 Multi-step homing mechanism for T_{eff} cell recruitment to the skin. T_{eff} cells primed by Ag in regional LN draining skin (not shown) become imprinted with skin-homing molecules, among which include adhesive glycoproteins CLA and CD43E, chemokine receptors CCR4 (Th2) and CCR10 (Th22), and integrins LFA-1 and VLA-4. (Step 1) Circulating T_{eff} cells in postcapillary venules of the dermis tether and roll in blood flow via engagement of CLA with E/P-selectins and CD43E with E-selectin. These interactions, which slow T_{eff} cell velocity thereby prepping cells for step 2, are facilitated by the tetrasaccharide moiety, sLe^X, which is synthesized by α1,3FT during skin imprinting. Of note, non-inflamed dermal endothelium constitutively expresses low levels of E-selectin, VCAM-1, and ICAM-1, all of which can be elevated in response to cytokine insult, thereby permitting T_{eff} skin-homing under both resting and inflammatory conditions. (Steps 2–3) Chemokine CCL17, which is secreted by Langerhans cells and keratinocytes in the epidermis and by fibroblasts and endothelial cells in the dermis, as well as CCL27, which is secreted by keratinocytes, bind T_{eff} cell-CCR4 and CCR10 receptors, respectively. CCL17 and CCL27 may be concentrated on glycosaminoglycans (GAG) expressed on endothelial apical, basal, or basement membrane surfaces to allow for enhanced chemokine receptor binding. Chemokine ligation of CCR4 and CCR10 elicits $G\alpha_i$-signaling, switches VLA-4 from an intermediate to highly active structure and LFA-1 from inactive to highly active, and eventuates in VLA-4/VCAM-1 and LFA-1/ICAM-1-mediated firm adhesion (arrows; solid = known signaling, dotted = speculated signaling). Integrins may also undergo activation independently of chemokine receptor signaling via a ‘Step 2 bypass’ circuit involving bimolecular association of E-selectin ligands (ie, CLA) directly with VLA-4 (arrow). (Step 4) Firmly adherent T_{eff} cells undergo VLA-4/LFA-1 and CCR4/CCR10-mediated transendothelial migration into the dermis and then potential further recruitment into the epidermis.

immobilize and concentrate CCL19, CCL21, and CXCL12 chemokines on HEV luminal surfaces.[29,32] Conformational opening of LFA-1 enables heightened interaction with HEV-intercellular adhesion molecule-1 (ICAM-1) and ICAM-2, slowing T-cell rolling and eventuating in firm arrest (sticking).[32] The newly adherent T cells then migrate laterally along HEV surfaces in search of ‘exit ramps’ before undergoing rapid transendothelial migration (TEM) into paracortical T-cell zones within peripheral (pLN) and mesenteric (mLN) LNs.[5,6] CCL21-driven haptotactic (adhesive) or chemotactic gradients might also impart T-cell directional motility into LN upon CCL21 binding to extracellular matrix proteins (ECM) embedded within the HEV basal lamina, including collagen IV, fibronectin, and laminin.[30,33] T cells can potentially choose between two routes of TEM, paracellular (migrating between HEV cell junctions) or transcellular (directly penetrating the HEV cell cytoplasm), though the exact mechanisms require further clarification.[32,33] Of additional significance, integrin $\alpha_4\beta_7$ (LPAM) on naive T cells interacts with mucosal addressin cell adhesion molecule-1 (MAdCAM-1) found on microvessels of the lamina propria, and on HEVs of Peyer’s patches (PPs) in the small intestine and on mLNs, to mediate rolling adhesive interactions within these tissues.[5,6] Other contributors like Vascular Adhesion Protein-1 (VAP-1) on HEV’s, or CD44 on naive T cells, may also aid LN homing, though their roles *in vivo* are controversial.[6] Having entered the LN, naive $CD8^+$ T cells quickly upregulate CCR4 (CCL4, CCL5, CCL17 ligands) and CCR5 (CCL3-CCL5 ligands), and follow chemokine gradients towards DCs.[34]

If naive T cells are not stimulated by antigen (Ag), they migrate to cortical lymphatic sinuses, follow sphingosine 1 phosphate (S1P) gradients in exiting SLO through efferent lymphatic vessels, are then returned to the bloodstream through the thoracic duct, and can again engage HEV and recirculate throughout the SLO network in search of Ags.[29,34] The elucidation of the molecular basis of emigration from LN was greatly aided by discovery of the potent immunosuppressant and S1P receptor 1 (S1PR1) antagonist, FTY720 (fingolimod), which prevents T-cell LN exit by downregulating S1PR1 expression.[29] LN egress is prompted by elevation in

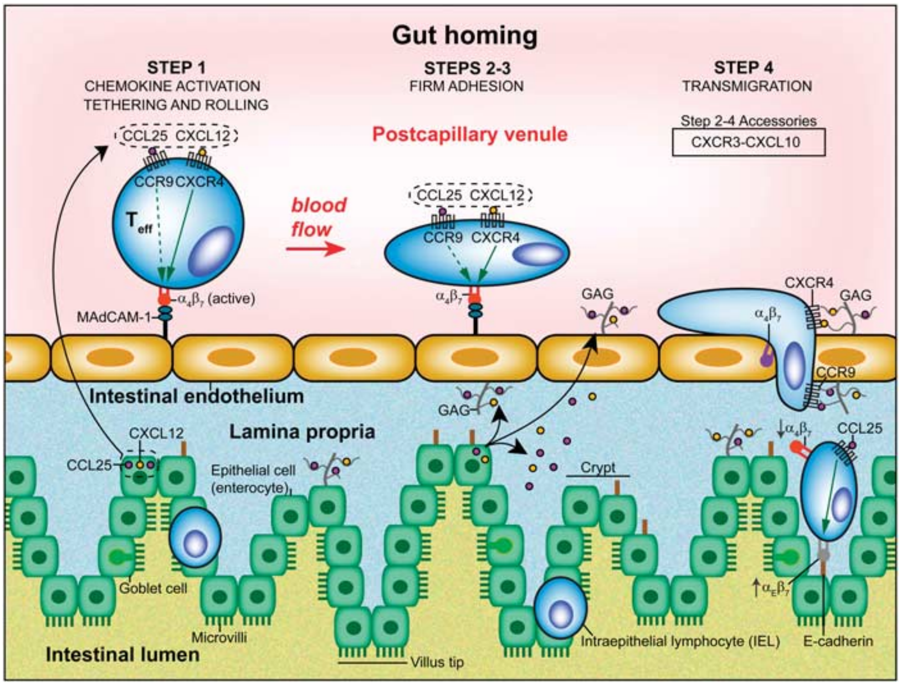

Figure 2 Multi-step homing mechanism for T_{eff} cell recruitment to the gut (small intestine). T_{eff} cells primed by Ag in Peyer's patches and mesenteric LN draining gut (not shown) become imprinted with gut-homing molecules, among which include chemokine receptors CCR9 and CXCR4 and integrin $\alpha_4\beta_7$.[16–18] (Step 1) Circulating T_{eff} cells in postcapillary venules of the gut (small intestine) engage CCR9-CCL25 and CXCR4-CXCL12 thereby eliciting $G\alpha_i$-dependent signaling, activation of $\alpha_4\beta_7$, and subsequent tethering and rolling on intestinal endothelial MAdCAM-1 (arrows; solid = known signaling, dotted = speculated signaling).[16–22] CCL25 and CXCL12 are produced constitutively by epithelial cells (enterocytes) of the small intestine while MAdCAM-1 is expressed constitutively in HEV of gut Peyer's patches and mesenteric lymph nodes (not shown) and by intestinal endothelium of the lamina propria.[16–19,21] (Steps 2–3) Rolling of $\alpha_4\beta_7$ on MAdCAM-1 eventuates in T_{eff} cell firm adhesion. (Step 4) T_{eff} cells undergo transendothelial migration into the lamina propria, facilitated by concentration gradients of immobilized CCL25 and CXCL12 on apical and basal endothelial GAGs, on epithelial GAGs, and within the lamina propria.[16–18,23] Accessory support of steps 2-4 may involve CXCR3-CXCL10 signaling (boxed).[16] A subset of T_{eff} cells traverse the lamina propria and then embed themselves as intraepithelial lymphocytes (IEL) into the epithelial cell layer of the intestinal lumen.[16,17] This latter process is accompanied by concurrently decreased $\alpha_4\beta_7$ and increased $\alpha_E\beta_7$ expression, CCL25-CCR9 signaling and activation of $\alpha_E\beta_7$ (arrow), and $\alpha_E\beta_7$ binding to E-cadherin.[16,18,24] During inflammatory reactions, the gut-homing repertoire is expanded to cause increased T_{eff} cell recruitment. This involves elevation in the expression of E/P-selectins, MAdCAM-1, VCAM-1, and ICAM on intestinal endothelium, along with CCR6-CCL20 signaling to increase T_{eff} cell adhesive interactions through selectin ligands, VLA-4, and LFA-1 (not shown).[22,25–28]

S1PR1-S1P signaling, which overrides G-protein ($G\alpha_i$)-coupled CCR7 LN-retention signals described above.[29] Conversely, CD69 binding to S1PR1 down-modulates S1PR1 expression and can inhibit T-cell exodus.[35] Notably, T-cell exodus can be induced independently of SRPR1-S1P signaling with pertussis toxin (PTX), an inhibitor of $G\alpha_i$ which mediates chemokine receptor signaling.[29]

Organ-Specific Imprinting and Homing of Activated T_{eff} Cells into Tissues

Naive T cells, which have recognized Ag displayed on the major histocompatibility complex (MHC) of mature DCs become activated (primed). To elicit priming, DCs uptake Ag at the infected tissue site, undergo maturation and lose expression of E-cadherin and of diverse chemokine receptors involved initially in peripheral tissue DC homing, upregulate LN-homing CCR7 and potentially CXCR4, and then rapidly transit through afferent lymphatic vessels or blood to T-cell areas of the draining LNs.[36] DC homing into the LN is orchestrated by integrin-activating cytokines such as LPS, TNF-α, and IL-1β as well as by gradients of CCL19, CCL21, and potentially SDF1.[36] Moreover, DCs extend long membrane folds called 'dendritic' processes that enhance the probability of T-cell capture, interaction, and priming.[36] Priming strength is fine-tuned by the duration and degree of T-cell receptor (TCR), co-stimulatory molecule (CD28 and others), and cytokine/chemokine stimulation, which help dictate programs of clonal expansion and differentiation into either short-lived effector (T_{eff}) cells or long-lived effector memory (T_{em}) and central memory (T_{cm}) T-cell subsets as delineated based on their distinctive phenotypes, functions, homing receptor repertoire, and trafficking patterns.[5–7,37] T_{cm} cells, in contrast to T_{eff} and T_{em} cells retain L-selectin and CCR7 expression and therefore recirculate primarily between blood and SLO.[34] Although T_{cm} cells can also upregulate tissue-specific homing molecules, including selectin ligands, CXCR3 and CXCR4, and may traffic to non-lymphoid organs such as skin and bone marrow; however, T_{cm} cells lack perforin or granzyme-based tumoricidal activities and do not exhibit the more robust peripheral tissue trafficking patterns characteristic of the effector cell subsets.[7,34] Thus, we focus below on the homing constituents specifically of the T_{eff} and T_{em} cell lineages, which we collectively refer to as T_{eff} cells, and which are of prime importance to cancer immunotherapy.

Activation of naive T cells coincides with differentiation into T_{eff} cells with concurrent loss of both basal L-selectin (via ADAM17-induced shedding) and CCR7 expression, and acquisition of tissue-specific homing molecules that, upon egress through the efferent lymphatic channel, enable vascular trafficking and entry into diverse tissues.[5–8] Downregulation of L-selectin and CCR7 routes T_{eff} cell homing to inflamed tissues by preventing migration back to uninflamed lymphoid organs. In parallel, DCs localized in draining lymph nodes molecularly 'imprint' specialized homing molecules onto T_{eff} cells present in those nodes, thereby fully committing and steering their trafficking back to the original tissue of DC Ag uptake.[7] Tissue-selective trafficking improves T_{eff} cell chances of re-encountering Ag. In elicitation of skin imprinting programs, DCs convert the inactive pro-hormone found preferentially in skin, Vitamin D3, to its active form, 1,25-dihydroxyvitamin D3, thereby inducing T_{eff} cell-CCR10 expression and driving epidermotropic migration that is responsive to keratinocyte-secreted CCL27.[38] Conversely, 1,25-dihydroxyvitamin D3 suppresses the T_{eff} cell gut-homing receptors, $\alpha_4\beta_7$ and CCR9, thereby enhancing skin-homing specificity. Similar metabolic processes help imprint T_{eff} cell acquisition of gut-homing markers, whereby DCs residing in PPs, intestinal lamina propria or mLN convert vitamin A to retinoic acid resulting in $\alpha_4\beta_7$ and CCR9 upregulation.[16–18,39] Hormone-independent means of gut imprinting involve Ag dosing and the OX40-OX40L co-stimulatory pathway.[39]

Imprinted, activated T_{eff} cells employ newly acquired chemokine receptors, predominantly CCR5 and CXCR3, in recognition of LN positional cues and in egress through efferent lymphatic vessels, ultimately entering the blood and utilizing their specific TCR plus specialized 'three-digit' zip code, comprised of unique selectin-chemokine receptor-integrin combinations, to enable organ-specific targeting (Table 1; Figures 1 and 2).[34,40] Induction of unique hierarchical assemblies of homing determinants is critical as diverse T_{eff} cell subsets and endothelial vessels may overlap in expression of homing guidance cues, for example in Ag relatedness, widespread presence of E-selectin, vascular cell adhesion molecule-1 (VCAM-1) and ICAM-1 on microvascular endothelial cells of skin, liver and bone, and in T_{eff} cell expression of LFA-1 and VLA-4 ($\alpha_4\beta_1$).[5,9,40–42] Indeed, all endothelial beds at sites of inflammation express E-selectin and VCAM-1, as these molecules are induced by inflammatory cytokines TNF-α and IL-1β.[43] Moreover, acquisition of T_{eff} cell phenotype coincides with increased expression of glycosyltransferases, principally FTVII, which confer generalized expression of sLe^X, the canonical E-selectin-binding determinant.[43] Characteristically, most T_{eff} cells also express the integrins LFA-1 and VLA-4, the receptors for ICAM-1 and VCAM-1, respectively. Thus *in vivo*, T_{eff} cells are endowed with the capacity to achieve step 1 tethering and rolling interactions and, upon LFA-1 and/or VLA-4 integrin activation, step 3 firm adherence on microvascular endothelial cells within inflammatory sites. Further evidence of redundant homing circuitry are humans (or genetically-manipulated mouse models) with the rare genetic syndromes of leukocyte adhesion deficiency (LAD) I or II, which exhibit universal defects in β_2 integrin (LAD I) or selectin ligand (LAD II) functional expression, respectively, coinciding with interference of immune cell migration into not only one but several tissue types and with increased risk of infection.[40,44] Sharing of homing pathways may help broadly distribute immune cells in scenarios where infection is widespread though may be overkill and potentially hazardous when inflammation is localized. Indeed, such capacity for widespread homing might be exploited in augmentation of T_{eff} cell trafficking in situations of broadly dispersed metastatic cancers as we suggest in Part III. But in conditions where restrictive homing is preferable as is generally so, or in the case of localized primary lesions, evolution has iteratively refined the homing code to tweak its specificity by engineering a hierarchical, customized catalog of T_{eff} cell selectin ligand and integrin adhesive proteins along with G-protein-coupled chemokine receptors. Chemokine receptor signatures are highly unique for a given cell type, dictated not only by a T-cell's imprinted predilection for a given tissue but also by its intrinsic cytotoxic ($CD8^+$) or helper ($CD4^+$) cell identity, eg, $CD8^+$ (Tc1, Tc2, Tc17) or $CD4^+$ (Th1, Th2, Th9, Th17, or Th22). These variables intermingle in procurement of the finalized $CD4^+$ and $CD8^+$ T_{eff} cell homing profile, which may include chemokine receptors CCR1-CCR6, CCR8-CCR10, CXCR1-CXCR6, CX3CR1, and CRTH2.[33,34,45–48] As but one example, IFN-γ-positive $CD4^+$ Th1 cells and $CD8^+$ Tc1 cells express high levels of E/P-selectin ligands, VLA-4, VLA-6 ($\alpha_6\beta_1$), CXCR3 and/or CCR5 and traffic better to inflamed peripheral tissues and tumors compared with $CD4^+$ Th2 cells and $CD8^+$ Tc2 cells preferentially expressing IL-4, IL-5, IL-13, CCR3, CCR4, and CD294 (CRTH2, prostaglandin D_2 receptor 2).[49–52]

Extra fine-tuning of homing potential and specificity is conferred by the CD3/TCR antigen recognition complex (signal 1), co-stimulatory molecules such as CD28 (signal 2), and corresponding cytokine signature (signal 3), which help localize T_{eff} cells to antigenically distinct tissues including tumors and, in response to crosslinking or Ag/cytokine-dependent signaling, directly activate LFA-1 and VLA-4 integrins to promote T-cell adhesion and migration.[53–55] In some cases, TCR-induced activation of LFA-1 and VLA-4 may occur independently of Gα_i signaling, thereby bypassing chemokine-directed homing without complete abrogation of tissue-specific targeting.[56–59] Crosslinking of CD44 via its ligand hyaluronic acid or via engagement to E-selectin by the

CD44 glycovariant known as HCELL (to be described in greater detail below) can also bypass chemokine signaling to activate VLA-4 adhesiveness. Such chemokine-independence, an underappreciated deviation from the conventional multi-step homing model, may be more common than first thought as activated T_{eff} cells treated with pertussis toxin can still undergo LFA-1 and VLA-4 binding and spreading on endothelium via a phospholipase Cγ signaling mechanism.[60] Similarly, crosslinking of P-selectin glycoprotein ligand (PSGL)-1 via P-selectin ligation can directly activate T_{eff} cell LFA-1 adhesion to ICAM-1 irrespective of chemokine stimulation.[61]

Additional reinforcement of tissue-homing selectivity is imparted by the heterogeneity of normal or malignant vascular endothelium among distinct organs or tumors. Homing typically occurs at postcapillary venules that can vary dynamically in spatial, temporal and level of adhesion molecule, chemokine, Ag, and TA expression, as well as in surface presentation of these homing determinants on diverse endothelial proteoglycans, extracellular matrices (ECMs), basement membranes, or MHC.[5,40] Although incompletely understood, endothelial cells may directly process and present Ag, including TA, on their MHC molecules and also express co-regulatory molecules such as ICOS-L, PD-L2, CD40, and OX40I to impact T_{eff} cell activation and trafficking.[62] Ag presented on endothelium was found to enhance transmigration of antigen-specific T cells without impacting rolling or adhesion while also inducing T-cell division at low efficiency.[62] This ability to control T-cell responsiveness and cytokine production without full T-cell activation has earned endothelial cells the title of 'semi-professional' antigen presenting cells.[62] In addition, chemokines may be released from endothelial vesicles stored beneath the plasma membrane at defined 'hot spots' of T_{eff} cell contact.[60] However, the overall complexity of this combinatorial circuitry underlying the strength and specificity of T-cell homing operations continues to raise profound questions even today and suggests heretofore undiscovered traffic-control mechanisms and accessory molecules beyond the classic TCR and three-digit code described above. In fact, emerging data has now implicated several immune checkpoint receptors, PD-1, CTLA-4, and T-cell immunoglobulin and mucin domain 1 (Tim-1) and potentially Tim-3, in homing-related functions as described by us and others.[5,7,53,56,63] A final consideration is that the vast majority of studies on immune cell homing to date have leveraged rodent models, which differ in many profound respects from humans in terms of selectin ligand glycosynthetic pathways as well as in selectin-selectin ligand, integrin and chemokine expression patterns, among others.[29,64–67] Nonetheless, extensive interrogation and dynamic visualization of native or adoptively transferred T_{eff} cell trafficking mechanisms by intravital microscopy, gene knockout models, time-lapse parallel plate, and microfluidic flow chambers, and transwells have cemented a general, conceptually-agreed model for the multi-step homing machinery of T_{eff} cells into inflamed tissue. This knowledge continues to expand and enable a contextual framework for the future immunotherapeutic enhancement of ACT_{eff} cell-tumor infiltration.

Homing to Inflamed Non-Lymphoid Organs

As discussed above, elevated expression of E-selectin, VCAM- 1, and ICAM-1 on microvascular endothelial cells occurs at all inflammatory sites, resulting from TNF-α- and IL-1β-induced transcription of corresponding mRNA transcripts within hours of stimulus. Importantly, at sites of metastasis, these inflammatory cytokines are released by cells of the reticulo-endothelial system that are activated coincident with initial parenchymal invasion by cancer cells, thereby fueling endothelial display of E-selectin, VCAM-1, and ICAM-1.[68,69] In addition to cytokines, LPS can itself induce endothelial E-selectin, VCAM-1, and ICAM-1 expression.[70–72] Thus, as expression of E-selectin ligands is characteristic of many cancer types,[73] inflammation-related increases in E-selectin expression encourages tumor metastasis,[74] and there is evidence that expression of E-selectin may be prerequisite for creation of the 'pre-metastatic niche.'[75] Notably, neither E-selectin nor VCAM-1 are stored in intracellular compartments, however, the other vascular selectin, P-selectin, is stored in the Weibel–Palade bodies of endothelial cells (and in α-granules of platelets) and its surface expression can be rapidly upregulated via granular translocation (within minutes in endothelial cells and seconds in platelets) in response to inflammatory mediators like histamine and thrombin. Following surface expression on endothelium, P-selectin and E-selectin are both internalized by endocytosis; E-selectin is then degraded in lysosomes, whereas P-selectin is recycled to the trans-Golgi network and then returned to the Weibel–Palade bodies for subsequent re-mobilization.[76] In rodents and other non-primate mammals, in addition to upregulated vascular expression by granule translocation, P-selectin gene expression is also upregulated by TNF-α, IL-1β, and LPS. However, conspicuously in primates, *de novo* synthesis of P-selectin is not induced by any of these agents, as only the E-selectin promoter, not the P-selectin promoter, contains the requisite sequence response elements to transcription factors NF-κB and ATF-2 that mediate gene expression by TNF-α, IL-1β, and LPS.[77,78] Accordingly, in human immunobiology, recruitment of cells to inflammatory sites is predominantly dependent on E-selectin receptor/ligand interactions, whereas E- and P-selectin have overlapping roles in cellular recruitment in non-primate mammals.

T_{eff} cells primed by Ag in regional LN draining skin become imprinted with skin-homing molecules, among which include induction of several adhesive glycoproteins such as E/P-selectin ligands, LFA-1 and VLA-4 integrins, as well as CCR4 (Th2) and potentially CCR10 (Th22) chemokine receptors (Table 1; Figure 1).[5,6,50,79,80] Prominent T_{eff} cell E-selectin ligands include cutaneous lymphocyte Ag

(CLA), a specialized E-selectin-binding glycoform of PSGL-1, as well as a glycoform of CD43 known as CD43E.[81–86] CLA has been detected on 85% of T cells at sites of skin inflammation *in vivo* and $<5\%$ in inflamed, non-cutaneous sites, hence, its historically popular designation as a skin-homing receptor.[87–89] CLA bears the tetrasaccharide moiety, sLe^X, which is recognized by the HECA-452 mAb, and its biosynthesis is catalyzed in part by FTIV and VII, of which the latter enzyme can be induced by IL-2, IL-7, IL-12, TGF-β, Ag-priming, and promoter demethylation and is suppressed by IL-4 and retinoic acid.[40,81,90–94] Knockout mice lacking FTIV and FTVII fail to generate T$_{eff}$ cells that home to skin.[40] Skin inflammation upregulates cognate ligands on dermal post-capillary microvessels recognized by skin-tropic receptors, including E-selectin (in humans and other primates), and both E- and P-selectin (in non-primate mammals), chemokines CCL17 (CCR4 receptor) and CCL27 (CCR10 receptor), ICAM-1 (LFA-1 receptor), and VCAM-1 (VLA-4 receptor).[5,42] Non-inflamed skin microvessels also constitutively express low levels of the above factors in mice and humans, thereby permitting skin-homing under both resting and inflammatory conditions.[42] Operationally mimicking the step-wise migration of naive T cells under steady-state conditions as described above, CLA$^+$ T$_{eff}$ cells first tether and roll in blood flow on microvascular E- and P-selectins, undergo activation of their LFA-1 and VLA-4 integrins in response to CCL17-CCR4 and CCL27-CCR10 induced signaling, firmly attach and spread on endothelial ICAM-1 and VCAM-1, and then diapedese through the activated endothelial barrier, potentially via paracellular and transcellular routes.[40,95]

Elicitation of non-cutaneous homing often involves overlapping selectin/selectin ligand (eg, E-selectin-CLA) and integrin/integrin ligand (eg, VLA-4/VCAM-1) determinants to those outlined above for skin, especially during inflammation (Table 1).[5] However, some imprinted factors are more unique, thereby ensuring exclusivity in organotropic targeting. For example, restrictive T$_{eff}$ cell gut tropic mediators include $\alpha_4\beta_7$ (LPAM) that binds mucosal vascular addressin cell adhesion molecule 1 (MAdCAM-1) expressed constitutively on postcapillary endothelial venules and HEV of the small intestine (Figure 2) and colon.[7,16–19,33] Moreover, CCL25, the chemokine ligand of CCR9, is selectively expressed by epithelial cells of the small intestine though is absent from the colon.[7,18,96–98] Determinants targeting T$_{eff}$ cells to normal or inflamed liver, lung, or heart have been less well mapped in comparison to skin and gut (Table 1). Hepatotropic factors include CD44, VLA-4, and CCR5 on T$_{eff}$ cells and VAP-1 and CCL5 (CCR5 ligand) on liver sinusoids or vascular endothelium.[5,99] Lung predilection is conferred by T$_{eff}$ cell or airway mucosal-expressed CCR3-CCL28 and CXCR4-CXCL12, respectively.[5,100] Finally, cardiotropic accumulation is thought to involve CCR5-CCL4/CCL5, CXCR3-CXCL10, and hepatocyte growth factor (HGF).[5]

Homing to Inflamed Lymphoid Organs

As noted above in the steady-state, T$_{eff}$ cells are largely restricted from HEV-mediated LN access by virtue of having lost L-selectin and CCR7 expression, though these may remain on a small fraction of T$_{eff}$ cells enabling some recirculation back to LNs for Ag immunosurveillance.[47,96] This exclusion, especially of cytolytic CD8$^+$ T$_{eff}$ cells from LNs, reduces inadvertent killing of Ag-presenting DCs and preserves their ability to trigger primary and secondary immune responses. However, fever, inflammation, or hypothermia from infection, cancer or assault greatly expands the size and cellularity of draining LNs as Ag's undergo rapid transportation from peripheral tissues to LN DCs for presentation to entering T cells.[29] These changes arise in part from cytokines either locally-derived or transported via lymphatic conduits, which prime the HEV network to increase homing molecules and T$_{eff}$ cell recruitment independently of CCR7.[29] Namely, upregulation of HEV luminal P/E-selectins, CXCL9/CXCL10 chemokines (by TNF-α), and ICAM-1 (by IL-6, TNF-α, and IL-1β), permit entry of T$_{eff}$ cells via tethering and rolling (on selectin ligands), chemokine receptor activation (by CXCR3), and adhesion (by LFA-1), respectively (Table 1).[29,32,33,46,101] Elevation in HEV CCL21 presentation increases extravasation of naive T cells.[33] Concurrently, T-cell egress is blocked through downregulation of T-cell-S1PR1.[29] Increased CXCR3$^+$ cytotoxic T$_{eff}$ cell numbers may help ultimately neutralize and dampen immune responses via direct killing of Ag-presenting DCs.[33]

T$_{eff}$ Cell Retention and Conversion to Resident Memory

Resolution of T$_{eff}$ cell immuno-trafficking responses in inflamed tissues as described above (Table 1) and even within tumor lesions coincides with microenvironmental reprogramming of some T$_{eff}$ cells into resident memory T cells (T$_{rm}$) via incompletely defined mechanisms.[5] T$_{rm}$ cells are retained and survive long-term in virtually all mucosal and barrier-type tissues as well as in peripheral, lymphoid and non-lymphoid organs and do not readily recirculate.[34] As progeny of Ag-experienced T$_{eff}$ cells, T$_{rm}$ cells lack L-selectin and CCR7, upregulate CD69 and integrin CD103 ($\alpha_E\beta_7$), and stand poised at a moment's notice to respond immediately to future infections via rapid and robust expression of chemokines.[5,34] CD69 inhibition of S1PR1 signaling due to CD69-induced internalization of S1PR1, in parallel with CD103 binding of E-cadherin, is thought to block egress and maintain T$_{rm}$ cells within peripheral tissues.[5] Enhancement of T$_{rm}$ cell retention and survival may involve the co-expression of collagen-binding T$_{rm}$ cell integrins $\alpha_1\beta_1$ in the epidermis and $\alpha_1\beta_1$ and $\alpha_2\beta_1$ in the lung.[5,39] T$_{rm}$ cell persistence is also aided by the pro-survival cytokines IL-15, IL-7, and TGF-β in skin, or IL-2 in lung.[5] As CD69$^+$CD103$^+$ T$_{rm}$ cells and diverse TIL subsets have been identified in melanoma and various tumor metastases, this has important implications for immunotherapeutic approaches.[102]

T_{eff} CELL HOMING TO SOLID PRIMARY AND METASTATIC TUMORS

Compared with the molecular homing models described in Part I, emerging data from animal tumor models, tumor-immune co-culture systems *in vitro*, and patient tumor tissue extracted *ex vivo* has now begun to validate that $CD8^+$ T_{eff} cells exploit and co-opt at least some and perhaps even most of the well-described 3-digit homing molecules, including selectin-chemokines-integrins, as well as TCR-TA recognition, in completion of the classic step-wise trafficking paradigm into target lesions of diverse cancers, including melanoma (Table 2; Figure 3).[9] So potent are these homing mediators, they have even been intrinsically hijacked by cancer cells, and possibly cancer stem cell subsets as hypothesized by us previously, in elicitation and potentiation of the metastatic cascade, a copycat process termed 'hematopoietic cell mimicry.[40,73,114] T_{eff} cell homing into tumor tissue is further facilitated by tumor peripheral and intralesional neoangiogenic microvessels, even HEV-like conduits, which provide T_{eff} cell access 'roads' into tumors, though also paradoxically promote tumor survival and dissemination. After infiltrating tumor tissue, $CD8^+$ T_{eff} cells must then physically contact tumor cells via recognition of TAs presented on tumor-MHC-I molecules and elicit rapid perforin/granzyme or slower Fas/Fas-ligand (FasL)-based elimination of tumor cells.[115–119] Nonetheless, significant hurdles preventing T_{eff} homing have become increasingly clear, whereby tumors disrupt and thwart TIL lesional penetrance through tumor-directed aberrancies of endothelial vessels and adhesion molecule expression, chemokine-chemokine receptor mismatching, immunoediting of TA expression, immunosuppression, and recruitment of cancer-associated fibroblasts. These disparities are thought to underlie the significantly reduced baseline entry of T_{eff} cells into tumor venules in comparison to diseased tissues of bacterial or viral infections, thereby contextualizing the TIL homing deficit.[120] Below, we explore these important considerations of the TIL homing paradigm and then summarize several dominant players.

Selectins, Integrins, and Other Adhesive Molecules

Several adhesive molecules have been correlatively or directly implicated in homing of $CD4^+$ and $CD8^+$ T cells into melanoma and into other tumor types (Table 2; Figure 3). In an *in vivo* model of established melanoma, adoptive transfer of $CD8^+$ T cells expressing a transgenic TCR specific for ovalbumin (OVA) (OT-I cells), and also harboring genetic deletions of FTIV and VII required for the synthesis of E/P/L-selectin ligands, more poorly infiltrated B16-OVA tumors in comparison with selectin-ligand$^+$ OVA-specific $CD8^+$ T cells.[40,103] Consistently, mAb blockade of thermally-upregulated E/P-selectins on B16-OVA microvessels, inhibited trafficking and corresponding tumor lysis by adoptively transferred OT-I cells.[104] Preferential expression of VLA-4 on adoptively transferred $CD8^+$ Tc1 *vs* Tc2 cells was associated with better Tc1 intracranial homing and therapeutic control of OVA-melanoma (M05) lesions, while trafficking was blocked either by α_4 (subunit of VLA-4) or VCAM-1 mAbs or by small interfering RNA-mediated silencing of Tc1-expressed α_4.[105] Similarly, $CD4^+$ Th1 cells, which express higher levels of VLA-4 and VLA-6 than $CD4^+$ Th2 cells, trafficked better into OVA-M05 tumors.[52] mAb blockade of VLA-4/VCAM-1 and LFA-1/ICAM-1 interactions significantly reduced adoptively transferred VLA-4$^+$ $CD8^+$ T_{eff} cell entry into B16 melanoma lesions grown either subcutaneously (s.c.) or intraperitoneally (i.p.).[9] Adhesive constraints were non-redundant, suggesting different non-overlapping roles for VLA-4/VCAM-1 and LFA-1/ICAM-1 interactions, respectively. TILs isolated directly from patient melanoma tissue and expanded *in vitro* expressed the activation marker CD69, variable levels of LFA-1 and VLA-4, and bound better to resting or activated HUVEC and to skin-derived microvascular endothelial cells (HMECs) in comparison to human peripheral blood T-cell controls.[89] The aforementioned TIL-endothelial adhesion was blocked by mAbs primarily against β_2 (subunit of LFA-1), to a lesser extent against β_1 (subunit of VLA-4), and when used in combination together or with E-selectin mAb, synergistically reduced binding to activated endothelium.

Similar selectin- and integrin-dependent TIL homing strategies have been identified in non-melanoma cancers. For example, upregulation of CD69 but no increase in LFA-1 or VLA-4 expression was found on TILs isolated from patient breast tumors *vs* resting peripheral blood lymphocytes despite enhanced spontaneous LFA-1 and VLA-4-dependent adhesion to osteoblasts and bone marrow-derived stromal cells (BMSC).[106] In this study, autocrine signaling by TIL-expressed CCL3 and CCL4 were implicated in the spontaneous activation of LFA-1 and VLA-4. TILs from human hepatocellular carcinomas (HCC) and colorectal hepatic metastases (CHM) also showed overexpression of CD69, reduced L-selectin, moderate to high though equal levels of either LFA-1, VLA-4, and $\alpha_4\beta_7$ compared with levels on peripheral blood leukocytes and low expression of α_M (subunit of Mac-1).[107] Under shear-dependent rotary conditions, TILs expanded *ex vivo* from HCC bound both spontaneously and better to vascular and sinusoidal HCC endothelial tissue sections than did peripheral blood leukocyte controls.[107] TIL adhesion was blocked by mAbs mostly against ICAM-1 and by mAbs targeting LFA-1 but not Mac-1, as well as by mAbs to VAP-1 and to a lesser extent VCAM-1, while inhibitory activity was enhanced when mAbs were combined.[107] Consistently, VAP-1-dependent TIL adhesion has been observed in several solid cancers.[110] Unfortunately, in some instances tumor microenvironments may thwart TIL homing and effector functions by downregulating lymphocyte integrin expression as was observed in the case of $CD4^+$ and/or $CD8^+$ TILs extracted from colorectal cancer tissue, which showed lower expression of LFA-1 and/or VLA-4 integrins and reduced T_{eff} cell binding to ICAM-1 and VCAM-1 in

Table 2 Adhesive mediators of T_{eff} cell homing into melanoma and other tumor types

T_{eff} cell molecule	Tumor molecule	Cancer type	References
FTIV, FTVII		Melanoma (B16-OVA)	40,103
	E-selectin, P-selectin	Melanoma (B16-OVA)	104
	E-selectin	Melanoma (patient); TIL binding to HMEC and HUVEC	89
VLA-4 (α_4 subunit)		Melanoma (M05-OVA)	52,105
VLA-4		Melanoma (B16)	952
VLA-4 (β_1 subunit)		Melanoma (patient); TIL binding to HMEC and HUVEC	89
VLA-4		Breast cancer (patient); TIL binding to osteoblasts and BMSC	106
	VCAM-1	Melanoma (M05-OVA)	52,105
	VCAM-1	Melanoma (B16)	9
	VCAM-1	HCC (patient); TIL binding to HCC endothelium	107
	VCAM-1	Colorectal (patient); TIL binding to purified VCAM-1	108,109
VLA-6		Melanoma (M05-OVA)	52
LFA-1		Melanoma (B16)	9
LFA-1 (β_2 subunit)		Melanoma (patient); TIL binding to HMEC and HUVEC	89
LFA-1		Breast cancer (patient); TIL binding to osteoblasts and BMSC	106
LFA-1		HCC (patient); TIL binding to HCC endothelium	107
	ICAM-1	Melanoma (B16)	9
	ICAM-1	HCC (patient); TIL binding to HCC endothelium	107
	ICAM-1	Colorectal (patient); TIL binding to purified ICAM-1	108,109
	VAP-1	HCC (patient); TIL binding to HCC endothelium	107
	VAP-1	SCCHN (patient); TIL binding to SCCHN endothelium	110
CD44v10		Melanoma (patient); TIL binding to melanoma cells; TIL migration	111
uPAR		HCC (patient), CHM (patient); TIL migration	112
CEACAM-1		Melanoma (patient); homophilic TIL binding to CEACAM-1 on melanoma cells	113

comparison to peripheral blood lymphocyte controls.[108,109] Suppression of VLA-4 and/or VLA-6 on $CD4^+$ or $CD8^+$ T_{eff} cells has been linked to hyperphosphorylation of STAT6 by IL-4.[51,52,105,121] In total, the above results indicate that CD69 upregulation is a hallmark of activated tumor-infiltrating $CD8^+$ T cells. Moreover, selectin ligands in combination with variably expressed but constitutively active LFA-1 and VLA-4 integrins, and potentially of VLA-6, synergistically mediate TIL adhesive rolling in flow, firm adhesion to tumor endothelial selectins, ICAM-1, VCAM-1 and VAP-1, followed by lesional entry via a classic step-wise homing paradigm. Data also implicates $CD8^+$ Tc1 *vs* Tc2 cells and $CD4^+$ Th1 *vs* Th2 cells in superior homing capacity and tumor-infiltrating potential due to upregulation of selectin, integrin, and chemokine factors.

Additional adhesive molecules implicated in T_{eff} cell-tumor homing have included an alternatively spliced variant isoform of CD44, CD44v10, which was detected on TILs extracted from primary human melanomas and found to mediate heterotypic TIL adhesion to melanoma cells and migration and invasion into ECM collagen gels independently of hyaluronan, selectin, or integrin involvement.[111] Although TILs isolated from HCC and CHM lesions did not express the $\alpha_V\beta_3$ vitronectin integrin receptor, they unexpectedly bound vitronectin and underwent transendothelial migration mediated by TIL-expressed urokinase-type plasminogen activator receptor (uPAR).[112] Immune co-regulators, conventionally viewed in regulation of homing-independent T-cell proliferative, effector, and homeostatic processes, are now known to directly impact T-cell migratory and trafficking behavior. Regulation of T_{eff} cell accumulation in tumors by co-stimulatory or co-inhibitory (immune checkpoint) receptors carries great significance for ongoing immunotherapeutic trials, especially those employing immune checkpoint antagonists and transgenic TCR-based adoptive therapy approaches. Ag or mAb crosslinking of TCR, CD3, or CD28-induced T-cell LFA-1 and/or VLA-4 activation, and increased adhesion and homing.[5,53–55] Ligation of PD-1 by PD-L1 suppressed T-cell motility, which could be subsequently reversed by therapeutic blockade.[122] Anti-CTLA-4 mAb prompted LFA-1-dependent T-cell adhesion to ICAM-1 as well as enhanced motility on

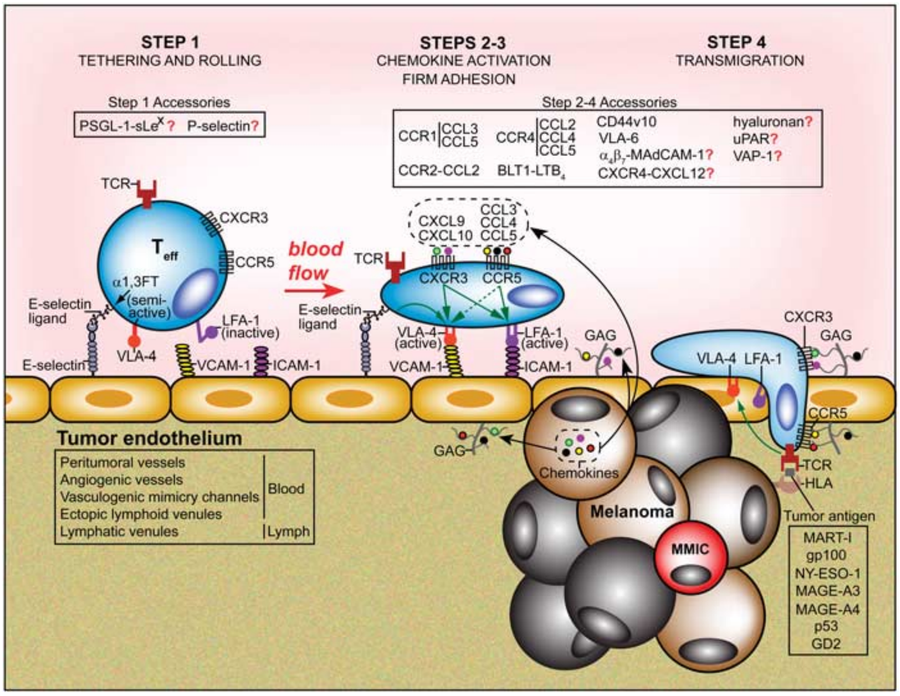

Figure 3 Native homing circuitry for T_{eff} (CD8$^+$) cell entry into melanoma and other lesional tissues. Melanoma-infiltrating T_{eff} cells natively express homing molecules at variable and suboptimal levels, including E-selectin ligands, VLA-4 and LFA-1 integrins, CXCR3 and CCR5-chemokine receptors, along with a TCR specific for a melanoma antigen. (Step 1) Circulating T_{eff} cells tether and roll in blood flow via engagement of undefined E-selectin ligands (synthesized by α1,3FT) with tumor endothelial E-selectin. This interaction slows T_{eff} cell velocity, thereby prepping T_{eff} cells for step 2. (Steps 2–3) Chemokines CXCL9, CXCL10, CCL3, CCL4, and CCL5 are secreted directly by melanoma and/or stromal cells of the tumor microenvironment and then bound by T_{eff} cell-CXCR3 and CXCR5 receptors. Chemokines may be concentrated on GAGs expressed on tumor microvessel apical, basal, or basement membrane surfaces for enhanced chemokine receptor binding. CXCR3/CCR5-chemokine ligation elicits Gα_i-signaling, switches VLA-4 from an intermediate to highly active structure and LFA-1 from inactive to highly active, and eventuates in VLA-4/VCAM-1 and LFA-1/ICAM-1-mediated firm adhesion (arrows; solid = known signaling, dotted = speculated signaling). Integrins may also undergo activation independently of chemokine receptor signaling via a ‘step 2 bypass’ circuit involving bimolecular association of E-selectin ligands directly with VLA-4 (arrow). (Step 4) Firmly adherent T_{eff} cells undergo VLA-4/LFA-1 and CXCR3/CCR5-mediated transendothelial migration and directly contact heterogeneous tumor cell subsets, including malignant melanoma-initiating stem (MMIC) and non-stem subsets, via TCR-based recognition of melanoma antigens displayed on HLA. Accessory homing mediators supporting T_{eff} cell infiltration into melanoma (boxed, no question marks) or other cancer types (boxed, question marks) are listed. Question marks indicate determinants which might be employed in T_{eff} cell trafficking into melanoma though for which direct data is lacking. Also listed are various blood and lymphatic channels that traverse the tumor parenchyma to provide access routes for circulating T_{eff} cells, including peritumoral and angiogenic vessels, vasculogenic mimicry channels, ectopic lymphoid venules, and lymphatic venules.

ICAM-1.[123,124] Tim-1, a mucin-like glycoprotein expressed on Th1 and Th17 but not Th2 T cells, mediated T-cell tethering and rolling on E/P/L-selectins and recruitment to the central nervous system in experimental autoimmune encephalomyelitis (EAE).[125] Whether or not Tim-1 as well as glycostructurally similar human family members, Tim-3, or Tim-4, enable T_{eff} cell homing into tumors requires further investigation. Thus, the impact of immune checkpoint blockade on TIL homing efficiency may represent an underappreciated variable for the optimization of immunotherapeutic approaches.

Conversely, some molecules viewed conventionally in the context of homing have been linked to homing-independent T_{eff} cell functions. Namely, L-selectin shedding from the surface of TA-activated CD8$^+$ T cells coincided with T_{eff} cell acquisition of oncolytic activities against melanoma as measured by CD107a (Lysosomal-associated membrane proteins, LAMP1) expression, a surrogate marker for cytotoxic degranulation.[126] Nonetheless, overall impact of T_{eff} cell L-selectin expression on tumor control is controversial given that adoptively transferred L-selectin$^-$ CD8$^+$ T_{eff} cells devoid of L-selectin and recognizing the melanoma Ag gp100 (or melanocyte protein, PMEL), expanded and controlled melanoma burden and lung metastasis with equal efficiency as compared with L-selectin$^+$ CD8$^+$ T_{eff} cells.[127] Carcinoembryonic Ag cell adhesion molecule 1 (CEACAM-1) was expressed on TILs isolated and expanded from primary and metastatic melanoma tissue, bound homophilically to CEACAM-1 expressed on melanoma cells, and inhibited T_{eff} cell-targeted killing and IFN-γ release.[113] In these cytotoxic assays, surviving melanoma cells showed upregulated CEACAM-1 underscoring its role in immunoevasion. PSGL-1 expression has been associated with reduced CD4$^+$ and CD8$^+$ T-cell proliferation, diminished TCR signaling, reduced effector cytokine secretion, and lowered responses to both viral infection and to melanoma via its induction of multiple immune checkpoint receptors, including PD-1 and Tim-3.[128] Conversely, PSGL-1 knockdown or mAb-ligation reversed suppression of T-cell proliferation and effector phenotype and thereby enhanced responses to viral infection and melanoma.[128] Whether distinct T_{eff} cell PSGL-1 glycovariants might differentially regulate selectin-dependent homing as opposed to selectin-independent effector activities has been proposed by us and requires further study.[63]

Chemokine Receptor-Chemokines

A number of correlative studies have linked intralesional accumulation of TILs to chemokine-chemokine receptor expression either on T_{eff} cells or within tumor locales.[48,129–133] CCR5 was the first chemokine receptor found to promote cytotoxic T-cell recruitment into tumors.[134] Since then, CXCR3 and its ligands CXCL9 and CXCL10, along with CCR5 (and its ligands CCL3, CCL4 and CCL5) have dominated the correlative findings of T_{eff} cell intralesional infiltration and favorable outcome in melanoma and colorectal cancer patients (Figure 3).[48,129–133,135,136] In these cancers and others, data has further implicated CCR1 (CCL3 and CCL5 ligands), CCR2 (CCL2 ligands), CCR4 (CCL2, CCL4, CCL5, CCL17, CCL22 ligands) in ancillary, more variable support of T_{eff} cell homing and disease free-survival (Figure 3).[48,129–131,135,136] Consistently, melanomas and colorectal carcinomas with low expression of chemokine ligands for CXCR3 and CCR5 are poorly infiltrated.[48,137,138] It bears mentioning that chemokines may orchestrate pleiotropic T_{eff} cell activities independent of and in addition to homing, for example, in mediation of T_{eff} cell proliferation, survival, retention, and egress, thereby underscoring the rationale for discriminating chemokine homing functions from other non-homing possibilities in consideration of immunotherapeutic strategies.[34] Another variable is that intralesional hypoxia, chemokines, and or other stimuli are known to downregulate (desensitize) chemokine receptor expression and signaling via endocytosis or may elevate their activities, arguing that oversimplified snapshots of chemokine receptor levels on TILs at one time point may obscure their temporally dynamic and hierarchical roles in tumor homing.[139] As an example of activities linked definitively to homing, a recent study found that chemokine levels in biopsies from patient melanoma metastases of the brain, lung, skin, and small bowel correlated positively with $CD8^+$ TIL numbers, these included CCL2-5, CCL19, CCL21, CXCL9-11, and CXCL13 but not chemokines CXCL12 and IL-8.[48] Selective upregulation of chemokine receptors CCR1, CCR2, CCR5, and CXCR3 and low levels of CXCR4 and CCR7 on $CD8^+$ T_{eff} cells *vs* naive cells was noted. $CD8^+$ T_{eff} cells migrated in response to tumor-derived supernatants of the M537 melanoma line expressing a highly diverse chemokine array, and the migration was blocked nearly completely by PTX, modestly neutralized by mAbs individually targeting CCL2-CCL4, and blocked even better down to near PTX levels with a mAb cocktail against CCL2-CCL5, CXCL9, and CXCL10. Thus, melanoma lesions express intrinsically variable chemokine signatures recognized by diverse T_{eff} cell chemokine receptors used in infiltration of tumors, among which primarily included four chemokine receptors and several melanoma-derived ligands, CCR1 (CCL3 and CCL5 ligands), CCR2 (CCL2 ligand), CCR5 (CCL3-CCL5 ligands), and CXCR3 (CXCL9 and CXCL10 ligands).

Consistently, another study found that metastatic melanoma-derived TILs expressed high CXCR3 and high though variable CCR5 depending on donor, intermediate CCR4, and low levels of CCR7 and CXCR1.[136] This profile mirrored the hierarchical expression on $CD8^+$ T cells derived from peripheral blood of healthy donors. Moreover, RT-PCR profiling of chemokine expression in 15 melanoma short-term cultures and in two melanoma lines identified CCL2, CCL4, CCL19, CXCL1, CXCL8, CXCL9, and CXCL12β. Upregulation of CXCL1 and CXCL8 and to a lesser extent of CXCL9 and CCL4 in nearly all melanoma samples *vs* melanocytes was observed. Remarkably, TIL migration towards melanoma-conditioned medium was associated with selective enrichment of CXCR1 (CXCL1 and CXCL8 ligands) and CXCR2 (CXCL1 ligand) at the TIL surface as opposed to their predominant intracellular localization prior to migratory assays.

Another highly detailed inquiry identified a $G\alpha_i$-coupled CXCR3 signaling mechanism in the homing of adoptively transferred $CD8^+$ T_{eff} cells into melanomas.[132] However, no evidence of CCR2 or CCR5 involvement was observed despite expression of complementary intratumoral chemokines, an observation possibly at odds with the findings above.[132] Namely, extracts from B16-OVA tumor implants contained high amounts of CXCL9, CXCL10, CCL5, and CCL2 as compared with non-inflamed normal skin, and $CD8^+$ T_{eff} cells from melanoma-bearing animals showed a $CXCR3^{hi}$ $CCR2^{int/lo}$ $CCR5^{int/lo}$ phenotype with concomitantly high migration to cognate CXCL9, CXCL10, CCL5, and CCL2. Migration *in vitro* was blocked by PTX or by genetic knockout of CXCR3, CCR2, or CCR5. As expected, experiments performed *in vivo* revealed 3-fold less homing of PTX-treated OT-I *vs* untreated OT-I cells to established B16-OVA tumors, thereby underscoring the requirement for $G\alpha_i$-coupled chemokine receptor signaling. CXCR3 neutralization, either by blocking mAbs or genetic deletion reduced T_{eff} cell accumulation in B16-OVA down to PTX-treated levels, with no involvement of CCR2 or CCR5 despite intratumoral presence of cognate chemokines. CXCR3 genetic ablation did not impact E/P-selectin ligand expression or consequent rolling of OT-I cells along tumor vessels, though did inhibit firm arrest despite no change in LFA-1 as was revealed by epifluorescence intravital microscopy. CXCR3 ligands, CXCL9 and CXCL10, while present on melanoma microvessel walls were not found on normal tissue, and mAb blockade of both reduced T_{eff} cell homing to melanoma.

Consistently, CXCR3 deficient OT-I cells homed ineffectively despite normal IFN-γ and granzyme B expression. Human CD8$^+$ T$_{eff}$ cells activated *ex vivo* had robust CXCR3 levels and highly variable CCR2 and CCR5 among individual donors. However, only CXCR3-mediated homing of human CD8$^+$ T$_{eff}$ cells *in vivo* to human M537 and M888 melanoma tumors as evidenced by CXCR3 mAb blockade or desensitization, despite the *in vitro* participation of CXCR3, CCR2, and CCR5 in chemotaxis assays. These data indicated a non-redundant role for CXCR3 in CD8$^+$ T$_{eff}$ cell trafficking in melanoma and provide a causal link underlying the efficacy of ACT$_{eff}$ cells in immunotherapy.

Finally, in murine models of cervical cancer and melanoma, the Gα_i-coupled receptor recognizing leukotriene B$_4$ (LTB$_4$), which is denoted BLT1 and has been identified on several immune subsets, was found to promote CD8$^+$ T-cell recruitment into tumors, diminishing lesional size and prolonging survival.[140] In contrast, BLT1 deletion did not impact CD4$^+$ TIL numbers. These results underscore the importance of diverse signaling receptors controlling both T$_{eff}$ cell homing and tumor burden.

Tumor Vasculature and Microenvironment

A vast network of blood and lymphatic channels traverses the tumor parenchyma, nourishing the hypoxic malignancy with vital oxygen and nutrients and also facilitating transport of TAs and DCs to draining LNs.[141–143] These dynamic fluid highways have been construed metaphorically as important gateways or checkpoints capable of both harnessing and hindering T$_{eff}$ cell infiltration.[104,132] Inasmuch, the tumor vasculature can be envisioned as a double-edged sword, in one respect offering hope as a highway access point for improving T$_{eff}$ cell targeting and overall immunotherapy while on the other hand providing tumor life support and 'get-away' exit ramps enabling metastatic escape, dissemination and cancer progression.

Ectopic lymphoid blood channels

Ectopic, tertiary lymphoid structures (TLS), HEV-like venules, and lymphoid chemokines have all been detected in both primary and metastatic tissue of several tumor types, including melanoma and others.[9,144–146] These tumor TLS mimic and recapitulate several structural aspects of their related secondary lymphoid organ relatives in terms of organization of B, T, and Ag-presenting cells segregated into distinct zones.[9] Moreover, unlike the cuboidal morphology of mature HEVs in LNs, tumor HEVs may be less differentiated, flat and/or express lower levels of PNAd.[144] Nonetheless, the presence of intralesional PNAd$^+$ HEV-like structures, MECA--79-reactivity, and/or expression of lymphoid chemokines CCL21, CCL19, or CXCL13 can promote recruitment of naive T cells and has also been positively correlated with intralesional T$_{eff}$ cell density, accumulation and prognosis.[9,133,144,146] These *de novo* lymphoid-like structures not only enable naive T-cell infiltration but also offer a tumor-intrinsic venue for T-cell priming, reactivation and differentiation into cytotoxic T$_{eff}$ cells directly within the tumor while avoiding T$_{eff}$ cell redirection and consequent dilution in draining LNs.[145] Formation of tumor TLS and/or HEV channels can mirror the generative pathways of normal LNs in terms of DC-lymphotoxin β (LTβ) utilization.[9] Alternatively, cancer tissue HEV generation has been further linked to TILs, namely CD8$^+$ T and NK cell secretion of LTα_3 and IFN-γ and signaling through TNF-α and IFN-γ tumor endothelial receptors.[9]

Peritumoral blood vessels

Though HEV-like conduits noted above may comprise $<10\%$ of the total tumor blood vasculature, the overall circulatory network inside lesional tissue is dominated by arterioles, capillaries and postcapillary venules.[9,143] These vessels may be present either peripheral to (peritumoral) or formed *de novo* within (angiogenic) tumor cores. As surrounding peritumoral vessels may be derived from already pre-existing normal endothelium prior to tumorigenesis, they often better resemble the vasculature of normal tissues.[143] These high-quality peripheral endothelial cells are structurally well-supported by a pericyte sheath, differentiated, perfused, and may show equal or in some cases higher constitutive or stimulus-induced expression of adhesive homing molecules *vs* normal endothelium of the same tissue, particularly of E-selectin, ICAM-1, VAP-1, or neural-cell adhesion molecule (NCAM).[107,142,143,147–149] Moreover, levels of E-selectin in Merkel cell carcinoma, VCAM-1, ICAM-1, or VAP-1 in melanoma, hepatocellular, or pancreatic islet cell carcinoma, and MAdCAM-1 in colorectal carcinomas correspond to T-cell entry and intralesional frequencies.[9,104,107,150–155] Intravital microscopy and histopathological examination has revealed that the peritumoral vasculature supports the majority of T$_{eff}$ cell recruitment, limited mostly to along the tumor margins or stroma.[106,112,143,156] For example, TILs within bone metastases of lung or breast cancer were primarily localized to the tissue stroma between bone and tumor mass.[106] Disruption of perivascular T$_{eff}$ cell migration deeper into the tumor interior has been linked to either steric hindrance of dense tumoral tissue, absence of vascular channels throughout the tumor, or from suppressive structural and signaling cues of nearby stromal cells, including cancer-associated fibroblasts (CAFs), myelomonocytic cells, MDSCs, and tumor-associated macrophages (TAMs).[157,158] CAFs lying adjacent to tumor perivascular channels may thwart T$_{eff}$ cell infiltration via synthesis of heavily-packed ECM.[159] Tumor vessels may additionally inhibit T$_{eff}$ cell homing and confer immune privilege by upregulating FasL through paracrine signaling of VEGF-A, IL-10, and prostaglandin E2 (PGE2) to directly kill tumoricidal T cells.[160] As normal endothelial cells or tumors themselves may express additional mediators that can suppress or kill T$_{eff}$ cells, such as galectin-1, PD-L1, PD-L2, and IL-10 among others, it is possible that tumor-derived malignant vessels may co-opt identical immunoevasive strategies.[161]

Angiogenic blood vessels

In contrast to the ordered peritumoral vessels, lymphoid HEVs, and/or postcapillary networks of normal tissues described above, scanning electron microscopy and imaging approaches have shown that the neoangiogenic, hypoxic tumor vessels formed deeply within tumors are of lesser quality, lacking in pericyte numbers and support, disorganized, poorly perfused, leaky with intercellular gaps, exhibit lower shear stress and T_{eff} cell flux, antigenically distinct, and are pathologically dysfunctional in homing molecules (ie, adhesion molecule and chemokine) expression.[142,143,162] Destabilization of intratumoral vessel integrity may ensue from dense, overlaying lesional tissue, which can create biomechanical tension and alter blood flow.[142] These tumor-intrinsic microvessels, often detected with mAbs against platelet-endothelial cell adhesion molecule-1 (PECAM-1), generally express low to nil E-selectin, P-selectin, ICAM-1/2, VCAM-1, MadCAM-1, or VAP-1 as has been observed in metastatic melanomas, squamous cell carcinomas (SCCs) and/or tumors of various origin, thereby hindering leukocyte binding, homing and entry into the tumor core.[9,104,107,163–172] As one striking example, expression of ICAM-1, VCAM-1, and E-selectin were >100-fold higher in normal lung than B16F10 melanoma tissue.[169] Lowered E-selectin expression in melanoma and SCC, as well as of ICAM-1, VCAM-1, and VAP-1 in colorectal hepatic metastases have been associated with reduced $CD8^+$ T_{eff} cell homing.[9,107,170] Consistently, activated $CD8^+$ T cells roll poorly and rarely undergo chemokine-directed firm adhesion in colorectal carcinoma vessels as revealed by epifluorescence intravital microscopy.[104] An additional consideration is that levels of E-selectin, VCAM-1, MAdCAM-1, VAP-1, or others on the tumor vasculature may be heterogeneous with respect to intrinsic vessel location within the tumor parenchyma and also in relation to specific lesional type (HCC *vs* CHM), its anatomical location (s.c. *vs* i.p.), individual patient, as well as to overall host immunocompetence.[9,107] Neovascular channels are often anergic to pro-inflammatory cytokine insult (TNF-α, LPS, IL-1β) and to induction of leukocyte rolling, adhesion, and adhesive molecule expression.[104,165] Such dysfunction may arise in part from endothelin B-receptor upregulation, which on ovarian tumor endothelium, was found to retard ICAM-1 expression, T_{eff} cell adhesion, and TIL intralesional frequency, and also coincided with reduced survival.[154] Suppression of E-selectin, ICAM-1, and VCAM-1 can result from angiogenic factors such as VEGF and fibroblast growth factor (FGF), which are overexpressed by both tumors and tumor microvessels.[169,172] The tumor vasculature is antigenically distinct from normal endothelium and this fact has been exploited in the successful development of cancer vaccines targeting tumor angiogenic vessels as described in Part III.[162,173]

Vasculogenic mimicry blood channels

Melanoma cells and diverse tumor cell types may directly generate perfused vascular channels themselves independently of endothelial cell-based angiogenesis in an intriguing though ill-understood process known as vasculogenic mimicry (VM).[174,175] VM is present in only the most aggressive tumors and has been defined as tumor-lined vessels positive for periodic acid–Schiff (PAS) reactivity and negative for the endothelial marker CD31.[174] Other VM characteristics have included a primitive stem cell-like phenotype, ECM remodeling, and interconnectivity with the tumor microvasculature.[174] As VM conduits nourish tumors with blood and nutrients and provide pathways for tumor cell egress, VM has been associated in melanoma and various cancers with increased tumor invasion, metastasis and poor clinical outcomes.[174] Several regulators of VM have been identified, including hypoxia, galectin-3, and several signaling proteins.[174] Cancer stem cells have also been implicated in VM channel formation.[174] For example, in comparison with non-stem bulk tumor cells, $ABCB5^+$ malignant melanoma-initiating cells (MMICs) preferentially express vascular differentiation or endothelial growth markers, CD144 (VE-cadherin), TIE1, and VEGFR-1, and form laminin-positive VM channels in response to VEGF-induced signaling.[176] Whether VM conduits express adhesive and homing molecules, allow T_{eff} cell access, and are exploitable in improvement of ACT and immunotherapy is unclear.

Lymphatic venules

Nearly all vascularized tissues are also traversed by lymphatic endothelial vessels (LEV), with tumors being no exception. LEVs act as highways that unidirectionally funnel Ag and DCs from normal tissues or tumors into draining LNs via afferent venules.[177] Intralesional LEV density has been correlated with metastasis and poor prognosis.[177,178] Lymphangiogenesis is induced mainly by VEGF-C/D derived from tumors, stroma, and infiltrating myeloid cells.[177,179] Typically quiescent, LEVs may undergo remodeling or activation in response to inflammation and the tumor microenvironment. Though little is currently known about homing molecule expression on lymphatic endothelium in cancer, a recent report found upregulation of ICAM-1 and VCAM-1 on LEV in oral tongue SCC and a positive association with metastasis and poor prognosis.[180] Tumor cells lying adjacent to LEVs and expressing cognate integrin receptors adhere to LEV adhesion molecules either directly or through linkage with immune cells, and then undergo step-wise transmigration into lymphatic channels, metastasize to regional LNs, and disperse into the bloodstream via the thoracic duct.[180] The lymphatic endothelium may present TAs to $CD8^+$ T cells, thereby deleting tumor-reactive lymphocytes and generating an immune-privileged location.[180] Whether tumor LEVs can be leveraged in the promotion of T_{eff} cell infiltration and immunotherapeutic approaches requires further study.

Tumor-associated immune and stromal cells

Though $CD8^+$ T_{eff} cells are believed to dominate the overall TIL infiltrate within highly restrained lesions, additional immune and non-immune cellular subsets residing inside the tumor microenvironment can influence T_{eff} cell homing and immunotherapeutic outcomes.[181] These accessory infiltrates typically employ identical or overlapping T_{eff} cell trafficking constituents as described above. For example, memory T cells, which are broadly grouped into T_{cm} ($CCR7^+CD62L^+$) and T_{em} ($CCR7^-CD62L^{lo}$) subsets, have previously encountered TA, are highly persistent and less differentiated than T_{eff} cells, and upon secondary re-stimulation with TA can differentiate into T_{eff} cells displaying increased anti-tumor responsiveness.[181] Nonetheless, although T_{cm} cells have limited tissue-homing capability outside of LN trafficking, circulating T_{em} cells may express all requisite homing molecules, albeit at lower levels than T_{eff} cells, to enable T_{em} cell trafficking into peripheral, non-lymphoid tissues and tumors, among which include sLe^X-bearing E/P-selectin ligands, chemokine receptors CCR4, CCR5, CCR10, and CXCR3, and integrins VLA-4, LFA-1, and $\alpha4\beta7$.[182–184] Another consideration is that $CD8^+$ T_{em} cells express high levels of cytolytic granzymes though show reduced perforin amounts relative to $CD8^+$ T_{eff} cells.[181] Thus, it has been speculated that ACT bolus preparations incorporating both $CD8^+$ T_{em} cells of high persistence, longevity, and proliferative capacity in combination with T_{eff} cells of greater homing and anti-tumor cytotoxicity might improve long-term tumor control.[185]

Less obvious than $CD8^+$ T cells have been the contributions of $CD4^+$ T cells to cancer suppression. One explanation offered is that although some solid tumors have MHC class II, many show reduced or absent expression rendering tumors invisible to direct TCR recognition by $CD4^+$ T cells.[186] However, high frequencies of $CD4^+$ T cells of the Th1 subset in tumor tissues have been correlated with better prognoses, and when administered autologously, have exhibited durable responses in cancer patients.[186–188] Moreover, $CD4^+$ Th1 cells can orchestrate accessory support of $CD8^+$ T_{eff} cell anti-tumor cytotoxicity by enhancing recruitment of both $CD8^+$ T and NK cells, blocking angiogenesis, and differentiating into $CD4^+$ T cells expressing granzyme B and IFN-γ and with direct cytolytic activities ($CD4^+$ CTL).[189] Conversely, $CD4^+$ Th2 and Th17 cell subsets have been observed to promote and inhibit tumor progression dependent on context. That is, recruitment of eosinophils by the Th2 cytokines IL-4 and IL-13 is tumor-suppressive, whereas IL-5 is tumor-promoting.[189] Further, although chronic exposure to $CD4^+$ Th17 cytokines can aid cancer progression, Th17-driven acute inflammation may inhibit it.[189]

Additional $CD4^+$ cell subsets, including follicular helper T cells (T_{fh}) and T_{reg} cells also have prominent roles in immune responses to cancer. T_{fh} cells express the transcription factor B-cell lymphoma 6 (Bcl-6), surface markers CD44, CXCR5, inducible T-cell costimulator (ICOS), and PD-1, and secrete IL-21 yet have low to nil levels of non-follicular positional homing molecules such as PSGL-1, CD62L, CCR7, and S1PR1.[189,190] T_{fh} cells found either in secondary or ectopic, tertiary lymphoid organs of tumors described above critically aid selection, maturation, and survival of B cells and corresponding Ab production against TA or tumor neoantigens. Tumor-infiltrating T_{fh} cells may also generate effector cytokines that aid recruitment of diverse immune cell subsets involved in preventing tumor progression, and Tfh cells can help create intratumoral follicular structures correlating with positive prognoses.[189] T_{fh} cells show high plasticity in their ability to downregulate Bcl-6, CXCR5, and PD-1, upregulate IL-7 receptor, and migrate between germinal centers and follicles as well as to enter the blood as circulating, memory T_{fh} cells able to potentially home directly into tumor tissues.[189,190] Natural T_{reg} (nT_{reg}) cells develop in the thymus independently of cytokines, whereas inducible T_{reg} (iT_{reg}) cells arise outside the thymus in peripheral and/or diseased tissues such as mucosa-associated lymphoid tissue (MALT) as well as potentially in tumor microenvironments in response to cytokine-mediated differentiation.[191] Both nT_{reg} and iT_{reg} cells express CD25 and forkhead boxP3 (Foxp3), and depending on tissue tropism, express CCR4, CCR5, CCR6, CXCR3, and CXCR4 chemokine receptors for directing them into malignant tissues via recognition of tumor-expressed CCL22 and other chemokine signatures.[191,192] Expression of E/P-selectin ligands have also been detected on T_{reg} cells within inflamed tissues, and might help steer T_{regs} into inflamed tumor sites.[193] Differentiation and expansion of T_{regs} is promoted by TGF-β expressed by tumor or dendritic cells.[194] T_{reg} cells inhibit immune responses to cancer via multiple mechanisms, including through expression of immunosuppressive IL-10 and/or by reduction of $CD4^+$ and $CD8^+$ T-cell proliferation, cytotoxicity, effector functions, and IL-2 production.[195] As a result, T_{reg} cell depletion schemas have been efficacious in enhancing anti-tumor immunity.[196] Whether the T_{reg} cells described widely in diverse cancer settings are of the natural or induced type is largely unknown.

Tumors commonly hijack neighboring stromal cells to promote tumor cell proliferation, angiogenesis, invasion, and metastasis. These co-opted stromal cells originate most often from surrounding fibroblasts though may also derive either from neighboring pericytes, epithelial cells, endothelial cells, or other cell types via epithelial–mesenchymal transition (EMT) or endothelial-mesenchymal transition (EndMT) events.[197] All such stromal participants recruited into the service of nearby malignancies have been referred to interchangeably as either tumor-associated fibroblasts, cancer/carcinoma-associated fibroblasts (CAFs), or tumor/cancer-associated stromal cells (TASC/CASC).[197] CAFs are dysfunctional in their expression of pro-tumorigenic IL-6, IL-8, IL-1β, TNF-α, and CXCL12 inflammatory cytokines among others, matrix metalloproteinases, growth factors, and of microRNAs (miR).[197] CAF secretion of TFG-β promotes EMT and metastasis, enhances nT_{reg} and iT_{reg} cell differentiation and proliferation, and inhibits $CD8^+$ T_{eff} and NK cell cytotoxicity.[198] CAFs may also directly shape T-cell infiltration in multiple ways, for example through secretion of CCL5 to recruit T_{regs} expressing its cognate CCR1 receptor, by inhibiting $CD8^+$ T-cell homing via macrophage-dependent polarization of T cells towards Th2, by compartmentalizing CXCL12 within the tumor microenvironment to disadvantage T-cell recruitment, and by remodeling the tumor ECM so as to anchor T cells in stroma-rich regions thus thwarting T_{eff} cell penetration deeply into the tumor bed.[199]

Summary

As illustrated in Table 2 and Figure 3, the above data implicates T_{eff} cell-expressed E/P-selectin ligands, LFA-1 and VLA-4 integrins, CXCR3 and CCR5-chemokine receptors, and TCR as major inducers of TIL homing into melanoma and various cancers. T_{eff} cell-expressed FTVII and corresponding selectin ligand expression are increased by IL-12, TGF-β, and TAs, and are reduced by IL-4-STAT6 signaling, which also inhibits VLA-4, and VLA-6 expression. Meanwhile, CCL3 and CCL4 mediate spontaneous activation of LFA-1 and VLA-4 allowing TIL infiltration. Accessory support in some instances from VLA-6, CD44v10, and uPAR, and potentially as hypothesized from CD28, PD-1, CTLA-4, and Tim-1 aids T_{eff} cell homing to tumors. Ancillary T_{eff} cell chemokine receptors depend on tumor type and individual patient and may include CCR1, CCR2, and CCR4, as well as BLT1. Additional players implicated in homing-independent TIL activities include either L-selectin in acquisition of T_{eff} cell cytolytic activity, and CEACAM-1 and PSGL-1 in suppression of diverse T_{eff} cell functions. Finally, though cancer types preferentially secrete chemokines relative to normal tissue controls, such as CXCL1 and CXCL8 as is the case in melanoma, suboptimal surface expression of complementary CXCR1 and CXCR2 receptors on T_{eff} cells prevents efficient or maximal homing.

On the flip side regarding the tumor vasculature and microenvironment, this review underscores several pro-homing TIL factors, including the principal tumor microvascular adhesive partners, E/P-selectin, ICAM-1, VCAM-1, VAP-1, chemokines CXCL9 and CXCL10 (CXCR3 receptor), CCL3, CCL4, CCL5 (CCR5 receptor), TAs, and $PNAd^+$ (MECA-79 reactive) HEV-like venule formation arising from CCL21, CCL19, CXCL13, LTβ, LTα and IFN-γ. Accessory support of TIL infiltration depending on tumor type may also involve MAdCAM-1, chemokines CCL3 and CCL5 (CCR1 receptor), CCL2 (CCR2 receptor), and LTB4 (BLT1 receptor). Conversely, tumor inhibition of TIL infiltration coincides with downregulation of adhesion molecules via endothelin B receptor, angiogenic VEGF and FGF, suppressive CAF and TAM cellular subsets, endothelial FasL (via VEGF-A, IL-10, and PGE2), and diverse immunosuppressive molecules, including galectin-1, PD-L1, PD-L2, and IL-10. Hypoxia, galectin-3, and VEGF promote VM channels to facilitate tumor progression and metastasis. Inasmuch, therapies aimed at either accentuating the TIL pro-homing circuitry or at neutralizing its inhibitors will greatly improve cancer immunotherapeutic outcomes.

TRANSLATIONAL ENHANCEMENT OF T_{eff} CELL TUMOR HOMING

Rapidly advancing yet still incomplete knowledge of TIL homing molecules in conjunction with new data on tumor microvascular defects and tumor immunoevasive tactics noted in Part II offer great translational opportunities for enhancing both TIL and ACT_{eff} cell intralesional trafficking (Figure 4). These therapeutic strategies may be broadly segregated into those selectively targeting T_{eff} cells directly or delivered systemically to render the tumor vasculature and microenvironment more permissive to T_{eff} cell homing. We relate the research findings above to both ongoing and future strategies in the improvement of T_{eff} cell homing.

T_{eff} Cell Homing Strategies

TIL, TCR_{gm}, and CAR T cells

Fundamental to the optimization of ACT clinical outcomes is the requirement for blood-injected ACT_{eff} cells, whether unaltered or genetically modified, to home, penetrate, and then eradicate cancerous tissues. Ideally, ACT_{eff} cells would also migrate to and persist within sentinel LNs, thereby eliminating LN metastases and undergo effector re-stimulation by TA recognition.[143] Three principal types of tumoricidal ACT_{eff} cells have been employed in personalized ACT strategies, all of which take advantage of T-cell-TA recognition to enhance homing selectivity and tumor penetration, and include (1) TILs, (2) T cells modified genetically by viral transduction to express high-affinity tumor-specific TCRs (TCR_{gm}), and (3) T cells engineered by viral transduction to express high-affinity chimeric antigen receptors (CAR).[143,200,201] Both TILs and TCR_{gm} express a conventional MHC (HLA)-restricted α/β chain TCR enabling recognition of either surface or intracellular TAs (mutant or nonmutant), when presented as peptides on tumor cell MHC.[200,201] Isolation and expansion of tumor-specific, high-affinity TCR TIL subsets has been challenging though, thereby incentivizing the customization of TCR_{gm} and CAR T by gene transfer technologies. In contrast, CAR T cells express a non-MHC restricted Ag receptor, which excludes recognition of intracellular TAs and limits surveillance to intact Ag presented on the tumor surface. Advantageously, CAR T cells do not require TCR-HLA matching or HLA-Ag presentation, and are therefore 'immunized' against two major drawbacks of TCR-based (TIL and TCR_{gm}) therapies, first against the HLA downregulation common in tumor cells and second against HLA polymorphisms, which restrict TCR therapies to only a subset of patients, ie, those with HLA-A2 found in 50% of caucasians.[200,201] All three ACT_{eff} cell subsets have undergone iterative improvements over the years, as for example first-generation CAR T cells contained only ZAP70 and CD3ζ signaling components enabling cytotoxic though suboptimal activation signals, whereas third-generation CAR T cells have

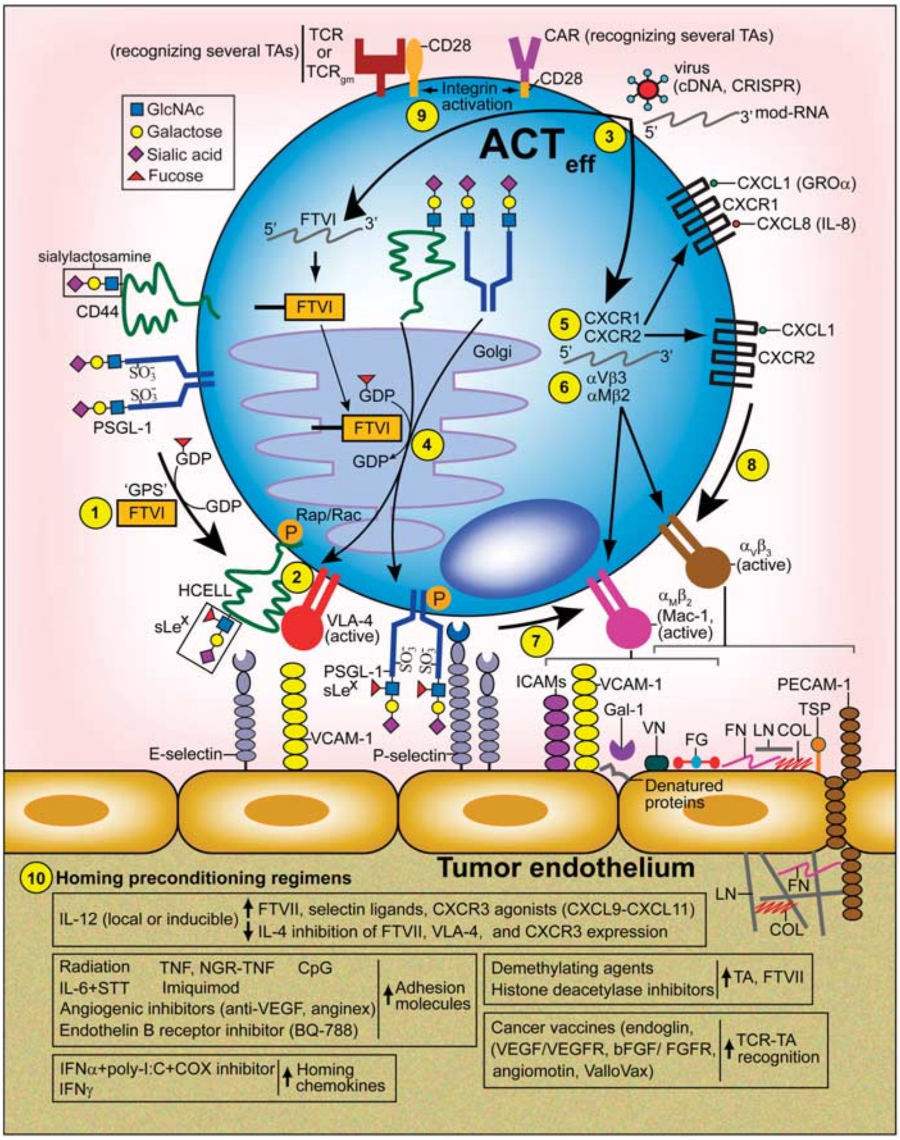

Figure 4 Optimization of ACT$_{eff}$ cells for broad delivery into widespread metastases (melanoma and others). Bioengineering of CD8$^+$ or CD4$^+$ ACT$_{eff}$ cells with vastly improved capacity for homing into widespread, metastatic tissues is now possible by combinatorially leveraging and integrating new glycoengineering and genetic engineering technologies with the latest knowledge on immune cell homing and cancer metastatic circuitries. As shown, suboptimal and/or minimal native glycosylation of CD44 and PSGL-1 on ACT$_{eff}$ cells could be compensated for using a (1) cell-extrinsic GPS approach with requisite α1,3FT (eg, FTVI or others) in generation of (2) E-selectin-binding CD44-sLe^X (HCELL) and E/P-selectin-binding PSGL-1-sLe^X (CLA) homing determinants. GPS may advantageously generate additional, unidentified selectin-binding glycoprotein and glycolipid homing determinants (not shown). Consequent bimolecular association of HCELL with VLA-4 via a Rap/Rac signaling mechanism, or of PSGL-1 with VLA-4 (not shown), would activate VLA-4 adhesion to VCAM-1 via a 'step-2 bypass'. (3–4) Cell-intrinsic creation of HCELL and CLA is shown, whereby viral transduction or transfection of mod-RNA, cDNA, or CRISPR-based platforms encoding α1,3FT (eg, FTVI or others) would result in its cytoplasmic translation, insertion into the golgi compartment, and heightened synthesis of sLe^X-selectin-binding moieties on CD44 and PSGL-1 (and possibly other glycoproteins and glycolipids, not shown) transiting the secretory pathway. Genetically introduced (5) CXCR1 or CXCR2, normally low or absent on ACT$_{eff}$ cells, would prime Gα_i signaling and homing responses when bound by cognate chemokines, CXCL1 or CXCL8, expressed by melanoma cells (or by other cancer types). (6) Genetic overexpression of $\alpha_V\beta_3$ or Mac-1 ($\alpha_M\beta_2$), also normally absent or low on ACT$_{eff}$ cells would, when rendered fully active potentially by (7) HCELL/ PSGL-1 'step-2 bypass' biomolecular association or by (8) CXCR1/CXCR2 chemokine receptor signaling, bind a plethora of diverse tumor endothelial adhesive proteins as shown. (9) Lesional targeting and homing specificity could be improved through positive selection and/or genetic overexpression of multiple different TCR, TCR$_{gm}$, or CAR receptors (and co-stimulators) recognizing diverse TA's and with capacities to activate integrins as shown. (10) Preconditioning regimens applied either prior to and/or following ACT$_{eff}$ cell infusion could synergistically enhance trafficking capabilities through augmentation of tumor endothelial or ACT$_{eff}$ cell pro-homing determinants, including adhesion molecules, chemokines and chemokine receptors, and TA. Incorporation of inducible-suicide genes (to limit ACT$_{eff}$-associated cytokine storms and inflammation), immune checkpoint blockers, and inhibitors of immune-evasive mechanisms could vastly improve immunotherapeutic outcomes in advanced cancer patients with widespread metastases.

now additionally incorporated co-stimulatory CD28, 4-1BB and/or OX40 to enhance proliferation, cytokine production, and survival.[201–203] Pre-clinical or clinical trials involving TIL or TCR_{gm} specific for TAs almost all in the context of HLA-A2, have included melanoma (MART-1, NY-ESO-1, gp100, MAGE-A3, MAGE-A4, GD2, p53), synovial sarcoma (NY-ESO-1, GD2), colorectal (CEA, NY-ESO-1, MAGE-A3), cervical (HPV16 E6, TROP-2), lung (NY-ESO-1, MAGE-A3, VEGFR2, and mesothelin) and breast cancer Ag (NY-ESO-1, TARP, PRAME, survivin, MAGE-A4, SSX).[200] CAR T cells have been employed in models or clinical trials of several leukemias expressing surface TA, such as chronic lymphocytic leukemia (CLL; CD19), acute lymphocytic leukemia (ALL; CD19), diffuse large B-cell lymphoma (DLBCL; CD19 or CD20), non-Hodgkin's and Hodgkin's lymphoma (CD30) and non-hematopoietic cancers such as neuroblastoma (GD2, CE7R), glioblastoma (Her2, EGFRvIII), colorectal (CEA), lung (Her2), breast (CEA), ovarian (folate receptor), and prostate (PSMA).[200,201]

Generalized therapeutic schemas have consisted of first isolating either TILs directly from autologous fresh, tumor tissue, or T cells from peripheral blood. Second, high-affinity TCR or CAR transgenes may be introduced through viral transduction, and then desirable T-cell subsets pre-selected, activated, and then expanded prior to re-infusion back into patients.[200,201] TIL, TCR_{gm} and CAR T cells have shown remarkable response rates in various cancer models and clinical trials. For example, third-generation CAR T cells recognizing a TA variant form of EGFR, EGFRvIII, found only on some tumors but not normal tissue, cured all mice with established intracerebral glioma.[204] A mixture of CAR T cells recognizing VEGFR2 found on the tumor vasculature in combination with TCR_{gm} against gp100 (PMEL), TRP-1 (TYRP1), or TRP-2 (DCT) melanoma Ag, synergistically eradicated established B16 tumors in mice and prolonged survival.[205] Additional CAR T-cell mixtures able to target both tumor cells and CAFs, which may comprise 90% of the entire tumor volume, have shown therapeutic promise and are poised for further development.[206–208] CAR T cells engineered to express heparanase, which degrades polymeric heparan sulfate, a potential barrier to T_{eff} cell homing into stroma-rich solid tumors, improved T_{eff} cell infiltration and anti-tumor activity via degradation of ECM components.[209] Utilization of TILs in phase I/II clinical trials have achieved response rates of up to 50%, including durable complete tumor eradication in some patients with metastatic melanoma.[210,211] Similarly encouraging responses have been observed in several clinical trials of TILs, TCR_{gm}, and CAR T cells.[185,210,212,213]

Despite showing remarkable promise in late-stage cancer models and clinical trials, ACT approaches require optimization and have come under scrutiny. Cerebral edema, neurotoxicity, and even death due to CAR T-cell induction of cytokine-release syndrome (CRS; also called cytokine storm) have plagued clinical trials and potentially delayed others.[214] These symptoms have been most pronounced in patients with the highest cancer severity. Composition and dosage of preconditioning regimens, cyclophosphamide, doxorubicin, vincristine, or prednisone, are thought to impact CRS.[214] CAR T cells may also induce a graft *vs* host like response when cross-reacting with identical or related Ag of healthy tissue arguing for affinity-tuned adjustments in CAR T-cell sensitivity for Ag and which has shown promise.[203,208,215,216] As a result, genetic engineering of inducible-suicide genes capable of triggering T-cell apoptosis at a moment's notice holds great potential in reducing CRS and adverse events.[203] An overarching hurdle has been that ACT requires a prohibitively high infusion number of ACT_{eff} cells exceeding a critical threshold to be therapeutically effective as the number that actually completes the homing cascade and infiltrates the tumor is impractically small. To give an idea, the concentration of Ag-specific $CD8^+$ T cells required to completely eradicate a 2×10^7 per ml concentrate of cognate Ag-expressing melanoma cells in collagen fibrin gels was $\geq 10^7$/ml of gel.[217] Another drawback is that in comparison with TCR_{gm} and CAR T protocols, TIL isolation and *ex vivo* expansion is more difficult, timely, and costly considering the low TIL numbers present within fresh tumor tissue and the careful expansion and screening phases needed to generate numbers of tumor-reactive TILs well into the billions required for therapeutic use.[213] Some malignancies may either lose or express nil levels of cognate TAs altogether because of antigenic drift arising from immunoediting and HLA downregulation, thereby resisting ACT targeting.[218,219] Most sobering is that individual tumor cells within even the same lesion exhibit distinctively diverse genetic profiles, thereby rationalizing for the targeting of multiple TAs concomitantly as has been reported.[220–222] Systemic preconditioning approaches to elevate TA expression as described below, in combination with iterative modification of tumor-reactive TILs, T_{gm}, or CAR T cells to combinatorial express multiple TCR and pro-homing integrins, chemokine receptors, cytokine/chemokines as discussed below could help greatly improve the homing efficiency and safety of ACT approaches, thereby reducing ACT_{eff} cell numbers, associated toxicity (cytokine storm) as well as costs.

Chemokine receptors

Chemokines CXCL1 and CXCL8 are secreted by melanoma cells in extremely high amounts in comparison with melanocytes, yet TILs derived from melanoma tissue express low surface (though intracellularly high) levels of cognate chemokine receptors CXCR1 (CXCL1, CXCL8 ligands) and CXCR2 (CXCL1 ligand).[136] Promisingly, ectopic though suboptimal overexpression of CXCR1 in TILs by RNA electroporation resulted in significant improvement of chemotaxis toward melanoma-conditioned medium and with no observed impairment of cytotoxic potential.[136] Similarly, lentiviral-based transduction and overexpression of CXCR2 in TCR_{gm} (pmel-1) T cells, which recognize the gp100 TA in the context of H-2Db, showed enhanced homing *in vivo* to MC38 colorectal carcinomas natively expressing CXCL1 along with better tumor regression and survival compared with control T cells.[223,224] Enhanced tumor regression and survival were also observed when CXCR2-transduced pmel-1 T cells were transferred into mice bearing CXCL1-transduced B16 tumors compared with control pmel-1 T cells.[224] T cells overexpressing CXCR2 by retroviral transduction showed increased IFN-γ production when incubated with CXCL1 *vs* control cells, underscoring the potential of chemokine receptor signaling to elevate both homing and effector anti-tumor activities concurrently.[224] Engineering ACT$_{\text{eff}}$ cell overexpression of additional chemokine receptors requires further investigation.

IL-12

Accentuation of homing could also involve IL-12, which has pleiotropic anti-tumor and pro-migratory activities as a potent inducer of FTVII and selectin ligands and of intratumoral CXCR3 chemokine agonists, including CXCL9-CXCL11, which promote CD8$^+$ T$_{\text{eff}}$ cell recruitment.[92,93,121,225] IL-12 can also overcome IL-4-mediated silencing of VLA-4 and potentially of CXCR3 expression, accentuates Th1 responses and Ag presentation, inhibits T$_{\text{reg}}$ cell functions, and reprograms MDSCs.[121,226] Nonetheless, constitutive or systemic IL-12 administration is severely toxic and can suppress T-cell proliferation.[226] However, IL-12 injected either locally into tumors or expressed in TA-specific T cells under the control of an inducible nuclear factor of activated T cells (NFAT)-responsive promoter system has been well-tolerated and shown remarkable efficacy in models of melanoma, ovarian cancer and leukemia.[226] This innovative, inducible NFAT system is activated upon TA stimulation, confines cytokine production to the tumor microenvironment, and allows for broader application of diverse cytokines that would otherwise be toxic if administered systemically. These successes have led to a phase I clinical trial, wherein adoptive transfer of NFAT-responsive IL-12-secreting TILs into patients with metastatic melanoma showed 34% or 63% objective response rates dependent highly on the total number of TILs infused, and requiring 10- to 100-fold lower numbers to achieve equivalent responses in comparison with genetically unaltered TILs.[227] However, toxicity, especially at high TIL numbers, included liver dysfunction, high fevers, and life-threatening hemodynamic instability likely caused from secreted IL-12. A related clinical trial using MUC-16$^{\text{ecto}}$-targeting CAR T cells modified to secrete IL-12 is underway for ovarian cancer along with a late-stage clinical trial involving intralesional electroporation of IL-12 cDNA into melanoma.[228,229]

Another exciting platform for restricting expression and localization of potentially toxic IL-12 to malignant tissue is the synthetic Notch (synNotch) receptor system.[230] This creative advancement employs T cells bioengineered to express an artificial form of the Notch receptor (synNotch) consisting of any extracellular antigen recognition domain of choice (eg, such as against CD19, Her2, etc.) fused to a cytoplasmic domain encoding any desired, artificially constructed transcription factor, such as Gal4-VP64. Binding of synNotch to its intended ligand, for example a cognate TA, activates the preprogrammed T-cell transcriptional circuitry and resultant delivery of its anti-cancer payload directly and selectively into the tumor microenvironment. This artificially constructed system, which is advantaged by its complete independence from T-cell native signaling mechanisms, allows for customized and diverse therapeutic responses, including in the control of defined T-cell anti-cancer cytokine profiles (IL-2, IL-12), effector functions, differentiation (Tbet and Th1 skewing), and macromolecule secretion (Abs against PD-1, CTLA-4) and has shown robust pre-clinical efficacy in tumor models.

Gene editing

Gene editing technologies have garnered recent excitement and are on the cusp of being leveraged to advance ACT-based immunotherapies. Among these, transcription activator–like effector nucleases (TALEN) and clustered regularly interspaced short palindromic repeats (CRISPR), provide innovative platforms for deleting endogenous TCR and HLA, thereby eliminating alloreactivity and reducing overall immunogenicity of donor ACT$_{\text{eff}}$ cells.[231] Genomic editing could also help optimize overall ACT$_{\text{eff}}$ cell functional capabilities via targeted disruption of genes that suppress T-effector activities and in parallel through insertion of transgenes that enhance homing, cytotoxic, and/or anti-cancer phenotypes. As for example, *de novo* expression or baseline elevation of integrin and chemokine receptors, in combination with targeted deletion of immune checkpoint receptors, either individually or together, could concurrently improve ACT$_{\text{eff}}$ cell homing, proliferative and effector responses to cancer. In particular, the recent generation of high-fidelity CRISPR-Cas9 nucleases exhibiting reduced off-target genome-wide effects and with improved safety represents a promising and exciting area of ongoing translational investigation.[232,233]

Exofucosylation and modified RNA

Standard conditions for culturing human lymphocytes (indeed, use of fetal bovine serum itself) dampen expression of E-selectin ligands.[81,234] Utilization of serum-free media boosts E-selectin ligand expression,[81,234] and TCR ligation in culture also modestly augments E-selectin binding.[81,234,235] Notably, although *in vitro* studies using mouse lymphocytes have shown that TCR ligation coupled with culture supplementation with IL-4 dampens E-selectin ligand expression,[225] incubation with IL-12[225] or TGF-β[91] or various other cytokines[235] significantly induces expression of FTVII and can also augment expression of other glycosyltransferases that direct synthesis of sLe$^{\text{X}}$, thereby resulting in marked increases in E-selectin ligand expression. However, the success of expansion of ACT$_{\text{eff}}$ cells *in vitro* could be compromised by cytokines used to induce glycosyltransferases that could result in cytokine-mediated undesired biologic effects, including polarization of cells, epigenetic changes, and alterations in cell viability. To overcome these shortfalls, we have developed two alternative approaches to enforce expression of E-selectin ligands based on glycosyltransferase-driven glycan engineering of sLe^X display: (1) Cell-extrinsic glycosylation via glycosyltransferase-programmed stereosubstitution (GPS); and (2) Cell-intrinsic glycosylation via transfection with modified mRNA (mod-RNA) that encodes requisite glycosyltransferase(s). With regards to the former, we have developed soluble α1,3FT's together with optimized reaction conditions to achieve highly efficient α(1,3)-fucosylation ('α(1,3)-exofucosylation') of the surface of viable cells.[236] The 'GPS technology' enforces expression of sLe^X determinants on cell surface glyoproteins and glycolipids that carry the requisite acceptor glycan known as a 'sialylated type-2 lactosamine' terminus: NeuAc α(2–3)-Gal β(1-4)-GlcNAc β(1-R); fucosylation of the N-acetylglucosamine (GlcNAc) within this trisaccharide core in α(1,3) linkage yields the canonical E-selectin-binding determinant sLe^X (NeuAcα(2–3)Gal β(1-4)[Fucα(1–3)]GlcNAc β(1-R). This approach has been used to generate E-selectin-binding activity on a variety of human cells, including hematopoietic stem cells,[237] mesenchymal stem cells,[238] neural stem cells,[239] and lymphocytes,[240] in each case conferring highly robust homing of cells to tissues whose endothelial beds express E-selectin. In a complementary strategy, we have *in vitro*-transcribed mRNA that encodes FTVI; this synthetic mRNA includes modified cytidine and uridine nucleotides (ie, modified RNA, 'mod-RNA') that help the mRNA elude host cell anti-viral defenses. Transfection of this mod-RNA enforces transient Golgi expression of FTVI, thereby engendering sLe^X decorations on scaffold glycoproteins and glycolipids, with resultant creation of E-selectin ligands.[241] In a direct comparison of extrinsic (GPS-enforced) and intrinsic (mod-RNA-enforced) fucosylation using human mesenchymal stem cells, we observed that both approaches yielded equivalently high E-selectin ligand expression, but there were marked differences in the kinetics and persistence of E-selectin-binding activity: exofucosylation yielded a 24–48 h duration of E-selectin binding, whereas mod-RNA allowed for a 5-day duration of binding activity. However, for purposes of enforcing sLe^X expression on lymphocytes for ACT indications, the GPS-based exofucosylation strategy would be more favorable as it avoids the need to achieve transfection-related cell manipulations (which requires electroporation in human lymphocytes) and it also avoids potential risks in introduction of nucleic acids and their product(s) into cells, including coincident induction of host viral defense responses and potential disruption of Golgi glycosylation networks.

A major advantage of enforced expression of HCELL, the E-selectin-reactive glycoform of CD44, on cell surfaces is that CD44 forms a bimolecular complex with VLA-4, and ligation of CD44 induces VLA-4 activation in the absence of chemokine signaling. From the very earliest observations of patients with congenital absence of β2 integrins (LAD I), it was recognized that these patients had, surprisingly, lesser deficits than expected in cell-mediated immunity.[242–244] Subsequent studies provided direct evidence that absence of β2-integrins did not impair LAD I lymphocyte binding to TNF-α-stimulated human endothelial cells.[245] Thus, it has been known for decades that endothelial adherence and transendothelial migration of lymphocytes can occur readily in the absence of LFA-1. In elegant studies in the early 2000s, Spiegelman and colleagues observed that crosslinking of CD44 on lymphocytes was sufficient to induce VLA-4 activation and transmigration of cells across TNF-α-stimulated endothelial monolayers in absence of chemokine input.[246,247] We explored the molecular basis of this effect using human mesenchymal stem cells, and found that engagement of CD44 triggers a Rap/Rac signaling-dependent upregulation of VLA-4 adhesiveness for its ligand VCAM-1, leading directly to transendothelial migration in the absence of chemokines.[248] We call this alternate migration cascade the 'Step-2 chemokine-bypass pathway' and it holds immense implications for the ability to direct lymphocyte trafficking to inflamed endothelial beds. Specifically, TNF-α induces expression of both E-selectin and VCAM-1 on microvascular endothelial cells, and, therefore, GPS-enforced expression of HCELL on lymphocytes (all of which constitutively express VLA-4) will prime trafficking of such cells to inflammatory sites; eg, HCELL engagement on E-selectin induces VLA-4 activation with subsequent lymphocyte firm adherence on VCAM-1 followed by extravasation. Thus, enforced expression of HCELL on the surfaces of ACT$_{\text{eff}}$ cells is a readily translatable roadmap for improving the delivery of systemically administered cells to sites where they are needed. Most importantly, the ability to improve localization of cells by enforcing E-selectin ligand expression, thereby enabling their tropism to E-selectin/VCAM-1-bearing endothelial beds, should allow for decreased numbers of infused cells needed to get an immunotherapeutic response, and, concomitantly, decreased numbers of cells needing to be expanded *in vitro*.

Systemic Elevation of Tumor Microvascular Homing Molecules

Induction of adhesive mediators

Sensitizing tumors for allowance of enhanced T$_{\text{eff}}$ cell infiltration could be accomplished through normalization and even reversal of adhesion molecule downregulation by various strategies. For instance, endothelial adhesive proteins in B16 melanoma and various tumor models have been upregulated in response to radiation therapy and angiogenic inhibitors, such as Anginex and anti-VEGF mAbs.[153,169,249] As VEGF has pleiotropic cancer-promoting properties in downregulation of tumor endothelial adhesion molecule expression, induction of neoangiogenesis, and recruitment of T$_{\text{regs}}$ and MDSCs, it has been a popular therapeutic target.[120] Subjection of B16-OVA melanomas or colorectal carcinomas to IL-6 and systemic thermal therapy (STT), whereby core temperature was raised to 39.5 °C ± 0.5 °C for 6 h, resulted in induction of E/P-selectin and ICAM-1 expression, promotion of $CD8^+$ T_{eff} cell rolling, adhesion and extravasation through tumor microvessels, and reduced tumor growth.[104] Systemic application of the BQ-788 inhibitory peptide against the endothelin B receptor, which is upregulated in the vasculature of diverse cancers, reversed endothelial ICAM-1 downregulation, increased T-cell-ICAM-1 endothelial adhesion, and augmented T-cell homing and cancer vaccine efficacy in models of ovarian and cervical cancer.[154] Treatment with the TLR7 agonist, Imiquimod, or TNF-α upregulated microvascular E-selectin and increased CLA $CD8^+$ T-cell recruitment in SCC.[170] TNF-α fusion peptides able to bind selectively to neoangiogenic vessels are also promising in that TNF-α fused to a Cys-Asn-Gly-Arg-Cys (NGR) sequence (NGR-TNF) bound a CD13 isoform on tumor endothelium, and even at low doses increased VCAM-1 and ICAM-2 levels, chemokine expression, T-cell homing, and improved cancer vaccine and adoptive immunotherapy in models of melanoma and other cancers.[120] Other TNF fusions, including TNF-RGR or a TNF-Ab variable peptide, are also under study.[120] The systemic application of CpG, a TLR9 agonist, induced ICAM-1 and VCAM-1 expression on tumor vessels.[151] Systemic triple cocktails of IFN-α, poly-I:C (TLR3 ligand) and cyclooxygenase (COX) inhibitors, which activated NF-κb selectively in both CAFs and infiltrating inflammatory cells, enhanced expression of T_{eff} cell-attracting chemokines, CCL5 and CXCL9-10, and suppressed local CCL22, a T_{reg}-attracting chemokine.[250] Pre-conditioning with IFN-γ-elevated intratumoral expression of three CXCR3 ligands, CXCL9-CXCL11, leading to increased T_{eff} cell homing.[251]

Nonetheless, a drawback of the preconditioning strategies above has been the paucity of angiogenic vessels present in some tumors, which renders the lesional environment resistant to T-cell infiltration irrespective of endothelial levels of adhesion molecules. Another limitation is that as described in Part II, tumors contain multiple types of perfused vascular channels, including VM, HEV-like and lymphatic vessels, several of which may be anergic to angiogenic-induced adhesion upregulation. Finally, both radiation and anti-angiogenic therapies have in some instances augmented tumor cell-intrinsic homing signatures and consequent invasion and metastasis.[252–255] These adverse side effects partly underlie the moderate or variable efficacy of conventional radiation and anti-angiogenic therapies and rationalize for implementation either transiently and/or at low doses, strategies which have proven efficacious in some tumor settings.[174,256]

TA normalization and cancer vaccines

As noted above, anti-tumor T_{eff} cell strategies depend on TCR recognition of unique TAs for optimal responses. Consistently, $CD8^+$ T_{eff} cells better infiltrate B16 melanomas engineered to artificially express a strong neoantigen, OVA, in comparison with the poorly immunogenic parental B16 line.[144] Other implantable tumor models have revealed similar findings.[257] However, many tumors are poorly immunogenic in part due to reduced TA-HLA expression as a means to evade TCR-targeted recognition and tumor elimination. For instance, highly immunogenic TAs found in melanoma and other cancers, like NY-ESO-1, are expressed often at low or nil levels due to epigenetic histone deacetylation or hypermethylation of the promoter.[258,259] Reactivation of TA expression and consequent responsiveness to adoptively transferred NY-ESO-1-specific TCR_{gm} lymphocytes has been accomplished with demethylating agents and histone deacetylase inhibitors.[258,260] Such TA normalization strategies could be combined with TIL and ACT directed approaches and tumor/tumor endothelial vaccines.[162,173] Some cancer vaccines have taken advantage of the upregulation of TAs on tumor angiogenic microvessels in comparison with normal endothelium. Accordingly, cancer vaccines targeting endothelial VEGF/VEGFR, bFGF/FGFR, $\alpha_V\beta_3$, angiomotin, and endoglin among others, have all shown success in pre-clinical or clinical trial cancer studies despite overlapping TA expression on normal vasculature.[162,173] Another exciting cancer vaccine, ValloVax, exploits the Ag rich profile found in highly proliferative human placental endothelial cells which approximates that of tumor endothelium.[162,173]

Immune effector and cytotoxic boosters

Systemic treatments that could improve immunotherapy independent of and/or in addition to induction of homing potential with lesser toxicity than IL-12 have included conventional IL-2 and more recently IL-7 or IL-15 therapies. These cytokines not only potentiate FTVII and selectin ligand expression but also act as adjuvants in cancer vaccine therapies and enhance anti-tumor T_{eff} cell responses through promotion of $CD4^+$ and $CD8^+$ T-cell activation, proliferation, survival, effector function, and/or differentiation into Th17 subsets.[90,261–265] Depletion of T_{reg} cells either by systemic administration of anti-CD25 mAbs or of IL-2-diptheria toxin fusion proteins prior to ACT infusion has had some success in partly controlling progression of melanoma and other cancers.[266,267] As TGF-β is one of the most potent orchestrators of tumor-immune evasion due to its suppression of T-cell proliferation, activation, and of release of cytotoxic factors, including perforin, granzyme A, granzyme B, FasL, and IFN-γ, strategies focused on interfering with TGF-β have garnered much attention.[268] Systemic neutralization of TFG-β or of its signaling pathways can restore T-cell-mediated tumor clearance.[268] Similarly, Galectin-1 (Gal-1) and other members of its β-galactoside-binding family, which are secreted by melanoma cells and various tumor types, tumor endothelium, and stromal cells, bind T-cell subsets to induce localized apoptosis, and/or skewing towards an immunosuppressive IL-4, IL-10, TGF-β, T_{reg} cell high tumor microenvironment.[269–272] Therapeutic suppression of T-cell Gal-1-binding determinants, with the metabolic inhibitor peracetylated 4-fluoro-glucosamine (4-F-GlcNAc), decreased

IL-10, increased IFN-γ and infiltration of tumor-specific cytotoxic T cells, and reduced melanoma growth.[271,272] Gal-1 activities were not limited only to TILs as its binding to melanoma-expressed MCAM-1 directly upregulated tumor cell adhesion and migration.[273] Interestingly, localized radiation therapy has shown promise in clearance of metastatic disease even in distant, nonirradiated regions via the abscopal effect, an incompletely understood, immune-dependent mechanism requiring further investigation.[274–276]

CONCLUSIONS AND FUTURE PERSPECTIVES

Cancer immunotherapy is an exciting, multidisciplinary arena holding unprecedented promise for late-stage cancer patients. Unlike most conventional systemic therapies suffering from toxicity and non-selectivity, for example chemo/radiotherapeutic regimens, T_{eff} cells are special in their capacity to home with high specificity to and penetrate nearly any anatomical space given the correct innate or engineered 'zip code', even in some cases entering previously discounted immune-privileged sites like the central nervous system, eyes, or testes.[226,277] This potent homing capability may be exploited to eradicate not only primary brain or testicular tumors in typically less-accessible sites but also widespread metastases. Cytotoxic T_{eff} cells can kill malignant targets within minutes, even in as little as five.[119] Leveraging these pre-existing evolutionary assets as they relate to profound T-cell homing and cytotoxic potentials will undoubtedly 'TIL't the balance towards exponential improvement of more efficient and safer cancer therapies able to synergize with clinically-approved immune checkpoint mAbs and others. Such ventures will require advancing mechanistic knowledge of the cellular and molecular components impacting T_{eff} cell traffic-control. In this review, we have attempted to encapsulate this knowledge as it relates to the promise as well as future challenges of cancer immunotherapy.

Pertinent for optimization of ACT_{eff} cell immunotherapeutic homing will be the delineation of T-cell subsets having the highest anti-cancer clinical activity. Namely, T cells in the earliest stages of differentiation (naive or central memory) have shown the greatest efficacy and persistence in ACT regimens as progressive terminal T-cell differentiation or exhaustion causes paradoxical loss of anti-tumor power through impairments in TCR signaling, and/or via reductions in either cytolytic activities, IL-2 and IFN-γ production, and adhesion, and/or entry into both pro-apoptotic and anti-proliferative programs.[278,279] Conversely, reduced differentiation may also coincide with lowered expression of tissue-homing molecules and trafficking potential. Thus, diverse T-cell subsets over a range of differentiation states may be optimal, as both speculated and evidenced by findings that $CD8^+$ T_{em} and T_{eff} cell cooperativity were needed for long-term tumor control in responding melanoma patients and that $CD8^+$ T_{cm} cells showed better anti-melanoma activity than did naive T cells.[185,280] Accessory help provided by tumor-specific $CD4^+$ lymphocytes is another consideration based on their noted presence in at least 20% of metastatic melanomas and well-recognized roles in orchestration of immune anti-tumor activities.[281] Finally, choice of $CD8^+$ Tc and $CD4^+$ Th cell type and respective ratios will also factor heavily in ACT bolus preparations. Thus, current ACT derivations involving T-cell isolation, subset selection, cell combinations/ratio utilization, and expansion require updating and revision to reflect these important considerations in the pursuit of ACT optimization.[278]

Equally prominent are questions pertaining to which homing molecules on T cells and cognate tumor and endothelial ligands should dominate therapeutic and bioengineering schemas. Comparative transcriptome and proteomics-based analyses of both homing molecule identity and expression on tumor *vs* normal endothelial vessels could prove useful in solidifying these candidates. As we have noted however, the multiplicity, variability, overlap, and redundancy of possible adhesive and signaling agonists of T cells and lesions are not just daunting and intimidating, but have also obscured their hierarchical and relative contributions. Therefore, understanding which imprinted homing molecules confer T_{eff} cell organotropic selectivity would offer therapeutic options for fine-tuning TIL and ACT_{eff} cell trafficking patterns to tissue-specific tumor venues (Tables 1 and 2; Figures 1–3). Conversely, patients with advanced, late-stage cancers exhibiting widespread metastases over multiple organs might benefit less from the compartmentalized homing strategies described above and more from unrestricted, broad dispersal into multiple tissues. Such pervasive homing might be accomplished as illustrated in Figure 4 via combinatorial upregulation of just a few of the most dominant and indiscriminate adhesive molecules known to date, among which include and we propose might involve HCELL, the most potent E/L-selectin ligand, PSGL-1, which when sulfated and heavily sialofucosylated recognizes all three (E/P/L) selectins, $\alpha_M\beta_2$ (Mac-1), a hematopoietic pro-adhesive/migratory integrin with extremely broad specificity for structurally diverse endothelial and ECM ligands, and/or $\alpha_V\beta_3$, although not natively expressed on T cells is commonly upregulated on a plethora of highly aggressive cancer types where it binds multiple endothelial ligands different from Mac-1 and facilitates metastasis.[40,282–288] Integrins of the β_2 subset are of particular significance as their principal cognate ligand, ICAM-1, is generally expressed on tumor endothelium at far greater levels than VCAM-1 or MAdCAM-1.[289] Moreover, HCELL, PSGL-1, LFA-1 (as well as the TCR and possibly TCR_{gm}), can prime integrin-induced stable adhesion and/or transmigration independently of chemokine signaling.[132] As expression of E-selectin dominates T-cell recruitment in humans (as opposed to in mice where P-selectin also contributes), HCELL might supersede P-selectin ligands like PSGL-1 in its ability to broadly disperse TIL and ACT_{eff} cells into metastases. Caution in augmenting PSGL-1 function is also warranted given one recent landmark study implicating it in master upregulation of multiple immune checkpoint receptors and in inhibition of pro-survival and effector CD4$^+$ and CD8$^+$ T-cell pathways.[63,128] Additional ACT iterations could incorporate accessory homing support not only from natively expressed and/or artificial elevation of both VLA-4 and LFA-1 but also from native or engineered variants of the TCR, TCR$_{gm}$, or CAR, from VLA-6, CD44v10, and uPAR, and unexpectedly from immunoregulators recently implicated in T-cell adhesion or migration, such as co-stimulatory CD28 and OX40 and co-inhibitory PD-1, CTLA-4, and Tim-1.[5] Inclusion of chemokine/chemokine receptors with preference for particular cancer signatures could prompt unrestricted homing to widely dispersed metastases as well as prime TIL and ACT$_{eff}$ cell integrin activation.

Regarding strategies to augment homing efficacy of ACT$_{eff}$ cells to melanoma lesions specifically, and considering that CD8$^+$ T$_{eff}$ cells innately already express some though variable levels of homing molecules, including though perhaps suboptimal levels of TCR, sLe^X-bearing PSGL-1, LFA-1, VLA-4, CXCR3, and CCR5, we hypothesize that genetic induction of diverse melanoma-reactive TCR's (and/or CAR) along with enforced expression of a more diverse repertoire of homing molecules such as HCELL, Mac-1, $\alpha_V\beta_3$, CXCR1, and CXCR2 on lesser-differentiated, more proliferative T$_{eff}$ and T$_{em}$ cell subset mixtures might provide superior, broad-based penetration and tumoricidal effects into widely dispersed lesions (Figure 4). Further enhancement of either TIL or ACT intralesional targeting could be prompted by systemic preconditioning with angiogenic inhibitors to normalize melanoma microvascular ligand expression and, at low doses or delivered transiently, might reduce likelihood of unwanted pro-metastatic side effects observed previously (Figure 4). Concurrent introduction of inducible-suicide genes into T cells would help protect against cytokine storms and other associated ACT pathologies. Combinatorial inclusion of inducible cytokines known to enhance T-cell homing and/or effector functions, such as IL-2, IL-7, IL-12, or IL-15, along with T$_{reg}$ cell and MDSC depletion regimens, immune checkpoint blockers, melanoma vaccines, and radiation therapy (abscopal effect) could synergize with engineered ACT$_{eff}$ cell trafficking constituents described above to further enhance widespread T$_{eff}$ cell homing and also aid T-cell proliferative and effector phenotypes. This combinatorial approach would afford a diverse menu of homing, effector, cytotoxic, and memory activities in realization of complete immunotherapeutic success against late-stage cancers. With greater consideration of these issues and with application of evolving technologies (eg, GPS) to alter expression/function of homing molecules, such customized pathway(s) may secure and help fully realize the curative potential of immunotherapy in malignant diseases.

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DISCLOSURE/CONFLICT OF INTEREST

According to the National Institutes of Health policies and procedures, the Brigham & Women's Hospital has assigned intellectual property rights regarding HCELL and GPS to the inventor (RS), who may benefit financially if the technology is licensed. RS's ownership interests were reviewed and are managed by the Brigham and Women's Hospital and Partners HealthCare in accordance with their conflict of interest policy. The remaining authors declare no conflict of interest.

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