Targeting cancer heterogeneity with immune responses driven by oncolytic peptides

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Abstract

Accumulating preclinical and clinical evidence indicates that high degrees of heterogeneity amongst malignant cells constitute a considerable obstacle to the success of cancer therapy. This calls for the development of approaches that operate – or enable established treatments to operate – irrespective of such heterogeneity. In this context, oncolytic peptides stand out as promising therapeutic tools based on their ability to drive immunogenic cell death associated with robust anticancer immune responses independent of intratumoral heterogeneity. Here, we review the main molecular and immunological pathways engaged by oncolytic peptides and discuss potential approaches to combine these agents with modern immunotherapeutics in support of superior tumor-targeting immunity and efficacy in patients with cancer.

Targeting a heterogeneous population of malignant cells

Intratumoral heterogeneity (ITH) is a broad concept referring to the genetic, epigenetic, transcriptional, phenotypic, metabolic, immunological and behavioral diversity of malignant cells originating from the same neoplastic lesion [1]. Indeed, at odds with early models reconducting human tumors to the purely clonal expansion of a genetically or epigenetically altered malignant precursor [2], modern technologies enabling an increasingly granular characterization of cancer cells and their microenvironment (*e.g.*, DNA sequencing coupled to multi-site biopsies, longitudinal single-cell RNA sequencing) revealed that developing neoplasms undergo considerable diversification [3-6]. This occurs not only as malignant lesions progress at different (micro)anatomical locations (spatial ITH), but also as they evolve over time, respond and potentially resist treatment (temporal ITH) [7]. Such a heterogeneity largely originates from the inherent genetic/genomic instability that characterize most (if not all) malignant cells coupled to (1) their elevated degree of functional plasticity, and (2) the relatively strong evolutionary pressure (manifesting with metabolic, trophic and immunological components) imposed by the tumor microenvironment (TME) [1, 8] (**Box 1**).

Thus, ITH is paramount for neoplastic lesions to progress locally as well as at distant metastatic sites despite the existence of numerous endogenous (*e.g.*, natural immunosurveillance) and exogenous (*e.g.*, anticancer therapies) barriers [9, 10]. Specifically, ITH generates a highly diverse pool of malignant cells that have a superior likelihood to survive a wide range of selective pressures as a population [1]. High degrees of ITH have been consistently associated with aggressive disease, resistance to treatment and poor outcome in a variety of oncological settings [3, 11, 12]. However, an elevated genetic diversity, such as that originating from defects in DNA mismatch repair (MMR), has also been linked to the generation of tumor neoantigens (TNA), which are key targets for tumor-specific $CD8^+$ cytotoxic T lymphocytes (CTLs)[13]. Accordingly, MMR-deficient tumors that develop so-called microsatellite

instability (MSI) exhibit superior sensitivity to immunotherapy with immune checkpoint inhibitors (ICIs) [14], although the emergence of specific clones with limited antigenicity or exquisite immunosuppressive properties, which is also enabled by ITH, may ultimately compromise the efficacy of treatment [15].

Importantly, cancer cells can only tolerate the alterations underlying ITH within a specific threshold, beyond which cellular fitness and/or the entire TME architecture may collapse [16]. Based on this notion, some efforts have been dedicated to the development of ITH-aggravating regimens for cancer therapy [17], so far with limited success. Indeed, while boosting ITH may actually cause the demise of some cancer cells that already display considerable genetic, epigenetic, transcriptional or metabolic rearrangements [18, 19], malignant cells with relatively milder alterations could benefit from this approach and achieve a competitive advantage that enables rapid disease progression [20]. Thus, ITH remains a considerable obstacle for the implementation of efficient anticancer therapies. Here, we discuss emerging data in support of using oncolytic peptides as therapeutic tools to target malignant cells despite ITH, as well as potential approaches to combine oncolytic peptides with immunotherapy for superior cancer control.

Molecular mechanisms of peptide-mediated oncolysis

Oncolytic peptides are a class of anticancer agents derived from or inspired by natural antimicrobial peptides (AMPs) that exhibit at least some degree of selectivity for malignant over normal cells (**Box 2**). Importantly, most oncolytic peptides mediate anticancer effects irrespective of genetic and epigenetic features of malignant cells, largely reflecting unique physiochemical properties that enable them to interact and disrupt lipid bilayers (**Box 3**). In particular, a net positive charge and a specific relative distribution of cationic and hydrophobic residues are key for various oncolytic peptides including bovine lactotransferrin (LTF)-derived [21], wasp venom-derived [22], silk moth-derived [23] agents and synthetic molecules like $(KAAKKAA)_3$ and SVS-1 [24, 25] to associate with membranes and engage in electrostatic interactions that promote lysis upon structural (re)configuration. Some degree of conformational flexibility and an elevated stability are crucial for efficient oncolysis by peptides, as demonstrated by numerous structure-activity studies involving amino acid substitution and/or redistribution [26-28].

Oncolytic peptides bind negatively charged cellular targets that are uniquely but homogenously displayed by cancer cells, which makes them suitable agents for eradicating tumors with high ITH. These molecules include phosphatidylserine, the major target of multiple oncolytic peptides including LTF-derived agents [29-32], phosphoinositides, which is selectively bound by human and plant defensins [33-36], glycosaminoglycans, targeted by dermaseptins [37], and gangliosides, which interact with buforins [38]. That said, some peptides display degree of selectivity for specific tumor types [39], likely depending on differences in cell membrane composition and electrochemical properties. Notably, a limited content of heparan sulfate [40, 41] and cholesterol [42] appears to enable superior lytic activity as these molecules limit the interaction of long peptides with plasma membrane. Moreover, some oncolytic peptides can interact with plasma membrane proteins that are overexpressed by cancer cells, and hence enable (at least

some degree of specificity), such as ATP binding cassette subfamily B member 1 (ABCB1) for the granulysin (GNLY)-derived peptide NK-2 [43]. Finally, so-called "masked" oncolytic peptides have been engineered for targeted activation only in the proximity of malignant cells, based either on local pH (which is relatively acidic in most solid tumors) [44] or on cleavage by cancer cell-derived metalloproteinases [45].

Upon association with the plasma membrane of cancer cells, some oncolytic peptides oligomerize and/or undergo structural rearrangements that enable rapid cytolysis and **accidental cell death (ACD)** [46] (see **Glossary**). Such a membrane-disrupting activity has been documented in human glioblastoma multiforme cells exposed to mastoparan-derived peptides [47] or a synthetic peptide known as LyeTx I-b, oral squamous cell carcinoma SCC15 and CAL27 cells treated with a LTF-derived peptide [48], human colon adenocarcinoma SW480 and Caco-2 cells responding to an engineered bacteriocin-derived peptide [49], human fibrosarcoma HT1080 cells treated with the synthetic 20-mer TH2-3 [50], a variety of chemosensitive and chemoresistant human cancer cell lines receiving the LTF-inspired peptide LTX-315 [51-53], multiple human bladder carcinoma cell lines exposed to the AMP magainin II [54], and various human lung carcinoma cell lines responding to cathelicidin derivatives [55, 56]. The ability of oncolytic peptides to permeabilize the membrane of (and hence kill) malignant cells more rapidly than most chemotherapeutics [51] has been shown to elicit robust growth inhibition (in the context of disrupted neoangiogenesis) in a variety of tumor xenograft models, encompassing models of sarcoma [57, 58] as well as breast [59, 60] and prostate [61] carcinoma. Importantly, pharmacological inhibition of **apoptosis** with the caspase blocker Z-VAD-fmk or regulated necrosis with the receptor interacting serine/threonine kinase 1 (RIPK1), necrostatin-1 (Nec-1) or the peptidylprolyl isomerase F (PPIF)-targeting agent cyclosporine A (CsA) failed to protect U2OS cells from rapid cytolysis driven by LTX-315 [52, 62], lending further support to the unregulated nature of cell death triggered by oncolytic peptides above a specific dose threshold.

At lower doses and/or in different cellular models, various oncolytic peptides can also trigger regulated forms of cell death that do not involve rapid permeabilization of the plasma membrane [46], but rather peptide translocation to the cytosol and interaction with one or more intracellular targets. **Mitochondrial outer membrane permeabilization (MOMP)** and consequent loss of respiratory capacity potentially coupled to activation of the intrinsic apoptotic pathway stand out as a major mechanism for the initiation of **regulated cell death (RCD)** by a variety of oncolytic peptides. These include LTX-315 [62] and other LTF-derived molecules [63, 64], the polycyclic AMP nisin Z from *Lactococcus lactis* [65], silk moth-derived AMPs and peptides thereof [23, 66-68], as well as TP3 and TP4, two AMPs derived from the Nile tilapia [69, 70]. Interestingly, while many of these peptides drive MOMP through BCL2-associated X protein (BAX) [71] upon accumulating in the matrix because of its electrochemical potential [62], a key role for early reactive oxygen species (ROS) generation and consequent activation of caspase 2 (CASP2) has been proposed for RCD driven by LTF-derived peptides [72]. According to this model, MOMP would be driven by CASP2 rather than by the peptides themselves. However, it seems that post-mitochondrial caspases including CASP9, CASP3 and CASP7 are not necessarily required for RCD driven by oncolytic peptides. Indeed, pan-caspase as well as caspase-selective inhibitors failed to protect malignant cells from LTF-derived peptides [52, 62, 63] and the cathelicidin antimicrobial peptide (CAMP)-derived peptide LL-37 or its analogs [73, 74], even though caspase activation was detectable in some settings. Thus, MOMP-dependent RCD driven by oncolytic peptides may also depend on caspase-independent mechanism including the activation of calpains and the nuclear translocation of apoptosis inducing factor mitochondria associated 1 (AIFM1) [73, 75]. Moreover, CASP8 activation has been mechanistically involved in the cytotoxic activity of MSP-4 (an α-helical cationic peptide from Nile tilapia) against human osteosarcoma MG63 cells [76] and dermaseptins against various human cancer cell lines [77].

Rather than directly targeting mitochondrial membranes, TP4 appears to mediate cytotoxic effects by interacting with solute carrier family 25 member 5 (SLC25A5, also known as ANT2), a component of the molecular machinery for **mitochondrial permeability transition (MPT)-**driven regulated necrosis and ATP synthesis. [46]. A similar mechanism, although potentially ANT2-independent, has also been suggested to account for the cytotoxicity of the bovine LL-37 homologs BMAP-27 and BMAP-28 [78]. Moreover, the synthetic oncolytic peptide DTT-304 triggered RIPK3- and mixed lineage kinase domain like pseudokinase (MLKL)-dependent **necroptosis** (yet another variant of regulated necrosis) [46] in multiple malignant cells [79], while the cytotoxicity of epinecidin-1 against fibrosarcoma cells and TP4 against glioblastoma cells could be hampered by the necroptosis inhibitor Nec-1 [80, 81].

Intriguingly, necroptosis induction in acute myeloid leukemia cells by the LTF-derived peptide PFR appears to depend on endoplasmic reticulum (ER) stress and increased ROS generation [82], demonstrating that membranous compartments other than the plasma membrane and mitochondria can be targeted by oncolytic peptides. Further, the β(2,2)-amino acid derivative LTX-401 as well as the LTF-derived peptide R-DIM-P-LF11-322 were found to interact with Golgi membranes in U2OS, human colorectal cancer HCT-116 cells and human melanoma A375 cells [30, 83, 84] as an early event before MOMP and RCD. Along similar lines, Brevinin-2R, a defensin isolated from *Rana ridibunda*, as well as multiple synthetic peptides including LTX-315, DTT-205 and DTT-304, were shown to associate with lysosomes in various cancer cell lines [79, 85]. Supporting an early mechanistic role for lysosomal targeting by oncolytic peptides, co-administration of the lysosomal inhibitor bafilomycin A1 diminished the cytotoxicity of DTT-205 and DTT-304 against U2OS cells [79].

Taken together, these observations indicate that oncolytic peptides largely operate by targeting membranous compartments (**Fig. 1**). However, alternative mechanisms of action including Ca^{2+} overload [86], altered microtubular dynamics [87], cyclin-dependent kinase 4/6 (CDK4/6) inhibition [88],

modulating of the extracellular matrix [70], and metabolic rewiring [89] have also been proposed for specific peptides. Whether these processes are upstream events in the cytotoxic pathways initiated by oncolytic peptides or rather bystander consequence of membrane disruption remains to be clarified.

Immunological effects of oncolytic peptides

A large body of evidence indicates that oncolytic peptides exert *in vivo* anticancer activity by promoting tumor infiltration by CTLs and other immune effector cells coupled to the depletion of immunosuppressive immune cells, hence resembling various clinically approved agents that inflame the TME (**Box 4**). At least in part, such an immunologically favorable therapeutic profile emerges from the capacity of various oncolytic peptides to elicit **immunogenic cell death (ICD)** [90], jumpstarting the so-called cancer immunity cycle [91]. Thus, cancer cells undergoing peptide-driven oncolysis emit a panel of chemotactic and immunostimulatory signals commonly known as damage-associated molecular patterns (DAMPs) as they release abundant antigenic material [90]. This culminates with the recruitment of antigen-presenting cells (APCs) or their precursors to the TME, the uptake of tumor-derived materials by APCs, APC migration to tumor-draining lymph nodes or tertiary lymphoid structures, and ultimately the priming of a tumor-targeting CTL-dependent anticancer immune response with local and systemic outreach [90, 92, 93]. The antigenic breadth of such response is generally high, implying that anticancer immunity driven by oncolytic peptides stands out as a promising tool to target tumors with elevated ITH.

Preclinical findings demonstrate that malignant cells succumbing to LTX-315, LTX-401, and the synthetic peptide RT53, emit the core set of ICD-relevant DAMPs [94], including the release of ATP, high mobility group box 1 (HMGB1) and mitochondrial components, the exposure of the ER chaperone calreticulin (CALR) on the cell surface, and the secretion of type I interferon (IFN) [95-98]. *In vivo*, such a DAMP profile is accompanied by increased immune infiltration, as shown in mouse MCA205 fibrosarcomas established in C57BL/6 mice [96, 98]. In line with this notion, intratumoral administration of LTX-315 to mouse B16 melanomas developing in C57BL/6 mice drove the upregulation of several pro-inflammatory cytokines such as interleukin 1 beta (IL1B), IL6 and IL18, culminating with tumor regression [51]. Similarly, intratumoral injections of LTX-401 induced complete and long-lasting

remission in multiple mouse cancer models established in immunocompetent syngeneic hosts [97, 99]. So did the administration of the synthetic peptide [D]-K3H3L9 in immunocompetent models of soft tissue sarcoma [58] and the intratumoral expression of defensin alpha 1 (DEFA1, also known as HNP-1) in immunocompetent mouse models of breast and colorectal carcinoma [100], although DAMP signaling was not characterized in these latter settings. Of note, LTX-315 also elicited effective CTL-mediated antitumor immunity in models of melanoma driven in mice by mutant Braf transforming gene (*Braf*) and phosphatase and tensin homolog (*Pten*) loss and soft tissue sarcoma elicited in mice by Kirsten rat sarcoma viral oncogene homolog (*Kras*) mutations and transformation related protein 53 (*Trp53*) loss [101]. Moreover, a recent case report indicates that LTX-315 was tolerated and induced tumor regression, coupled to increased CTL infiltration in a patient with a desmoid tumor of the thoracic wall [102]. Thus, various oncolytic peptides actively elicit, or at least do not inhibit, tumor infiltration by immune effector cells.

Importantly, local anticancer immunity driven by oncolytic peptides has been associated with systemic outreach, as demonstrated by growth retardation and/or tumor regression coupled to CTL infiltration in both treated and distant (untreated) lesions (in rat models of fibrosarcoma and hepatocellular carcinoma) [103, 104], and establishment of long-term protective immunological memory, as demonstrated in both vaccination and treatment settings (in rat models of hepatocellular carcinoma as well as in mouse models of lymphoma and fibrosarcoma) [79, 104, 105].

The immunotherapeutic activity of oncolytic peptides, however, may not be restricted to the activation of ICD. For instance, LTX-315 has been shown to deplete intratumoral $CD4^+CD25^+FOXP3^+$ regulatory T (T_{REG}) cells and myeloid-derived suppressor cells (MDSCs) [106], two population of cells with potent immunosuppressive effects [107, 108]. Of note, T_{REG} cell depletion by oncolytic peptides may originate, at least in part, from the permeabilization of cytotoxic granules and cytosolic leakage of granzyme B

(GZMB) [109]. Pardaxin (an AMP from *Pardachirus marmoratus*) promoted anticancer immunity in hamster models of oral squamous cell carcinoma by reducing the levels of immunosuppressive prostaglandin E2 (PGE_2) [110], while the bacterial AMP CSP32 favored macrophage polarization towards an antitumorigenic M1-like phenotype by boosting intracellular calcium signaling via the mitogen activated protein kinase (MAPK) cascade [111]. Finally, LL37 potently stimulated plasmacytoid dendritic cells (DCs) to secrete type I IFN by boosting extracellular nucleic acid uptake and detection via Toll-like receptor 9 (TLR9) [112-114]. Importantly, such activity culminated with superior type I IFN secretion by plasmacytoid DCs [112], which is a potent activator of natural killer (NK) cells [115]. Thus, NK cells may constitute additional immune effectors in anticancer immune responses driven by oncolytic peptides.

An LL-37 homolog from murine CRAMP also mediated chemoattracting effects on monocyte by favoring formyl peptide receptor 1 (FRP1) signaling [116], and targeted cancer-associated fibroblasts (CAFs) by altering their microtubular dynamics to limit tumor progression in an endogenous mouse model of colorectal carcinogenesis [117]. That said, the mouse analog of LL-37 has also been attributed with immunosuppressive effects downstream of 5'-nucleotidase ecto (NT5E, best known as CD73) overexpression and consequent accumulation of adenosine in the TME [118], polarization of tumor-associated macrophages towards an M2-like phenotype [119], and CTL apoptosis [120]. A similar immunosuppressive activity has been documented for human defensin β3, especially with respect to M2-like polarization [121] and cytotoxicity towards primary human monocytes [122]. However, defensin β3 also mediated chemotactic [123] and immunostimulatory [124] effects on immature DCs, suggesting that the net immunomodulatory effects of some oncolytic peptides may exhibit at least some degree of context dependency.

Taken together, these observations exemplify the ability of multiple oncolytic peptides to mediate therapeutically relevant immunostimulatory effects by inducing ICD as well as by favoring the reconfiguration of the TME toward an inflamed profile via both direct and indict effects on immune cells (**Fig. 2**).

Integrating oncolytic peptides in cancer (immuno)therapy

The bulk of data currently available on the anticancer effects of oncolytic peptides has been obtained in preclinical tumor models, most often human cancer cell lines maintained *in vitro* or xenografted in highly immunodeficient athymic (nu/nu) or NOD *scid* gamma (NSG) mice, exposed to oncolytic peptides as standalone therapeutic agents [47, 52, 54, 59, 82]. Thus, whether oncolytic peptides can be conveniently combined with other therapeutic modalities to achieve superior therapeutic efficacy in the context of preserved safety remains largely unexplored, with a few exceptions.

HX-12C, a synthetic derivative of an AMP from the Malaysian fire frog *Hylarana picturata* [125], reportedly synergized with the microtubular poison paclitaxel, the anthracycline doxorubicin and the platinum derivative cisplatin in the killing of chemoresistant human epidermoid carcinoma cells, largely reflecting the ability of HX-12C to inhibit chemotherapy efflux via ATP binding cassette subfamily B member 1 (ABCB1) [126]. Similarly, the synthetic peptide KLA cooperated with various death receptor agonists in the killing of cultured TRAIL-resistant LNCaP and PC3 prostate cancer cells *in vitro* [127]. An analogous cooperative cytotoxicity could be demonstrated between a derivative of the natural AMP melittin and the pyrimidine analog 5-fluorouracil against chemoresistant human hepatocellular carcinoma BEL-7402 cells *in vitro* [128], two peptides derived from the AMP pleurocidin and cisplatin against cultured human breast carcinoma MDA-MB-231 cells [129], a synthetic peptide containing *D*-residues (Amphipathic-*D*) and doxorubicin against multiple human prostate carcinoma cells [61], as well as TP4 and epidermal growth factor receptor (EGFR) inhibitors against various human non-small cell lung carcinoma (NSCLC) cell lines [130]. Moreover, the gonadotropin releasing hormone receptor (GNRHR)-targeted peptide EP-100 synergized with the poly(ADP)-ribose polymerase 1 (PARP1) inhibitor olaparib against a panel of human ovarian cancer cells lacking BRCA1 DNA repair associated (*BRCA1*) and BRCA2 DNA repair associated (*BRCA2*) mutations [131]. Of note, such a synergism could

also be documented in athymic female mice xenografted with human ovarian carcinoma HeyA8 cells [131].

Mastoparan (an AMP from the bee venom) synergized with gemcitabine in the control of mouse mammary carcinoma 4T1 cells established in immunocompetent syngeneic hosts, correlating with potent lytic activity against various cancer cell lines (but not peripheral blood mononuclear cells) *in vitro* [132]. Nisin Z considerably improved the ability of 5-fluorouracil to induce the apoptotic demise of cultured human squamous cell skin carcinoma A431 cells [133], and it synergized with systemic 5-fluorouracil or doxorubicin in immunocompetent BALB/c mice bearing squamous cell skin tumors driven by 7,12-dimethylbenz(*a*)anthracene (DMBA) alone or combined with 12-*O*-tetradecanoylphorbol-13-acetate (TPA)-driven [133, 134]. Such an improved therapeutic effect was accompanied by the modulation of multiple genes involved in apoptotic cell death, including the upregulation of *Trp53* and *Bax* and the downregulation of B cell leukemia/lymphoma 2 (*Bcl2*) [133] as well as by increased positivity for biomarkers of apoptosis *in vivo* [134].

Systemic doxorubicin could also be conveniently combined with intratumoral LTX-315 to achieve superior therapeutic efficacy associated with frequent tumor regression (in the absence of systemic signs of toxicity) against mouse triple-negative breast cancer 4T1 cells orthotopically implanted in immunocompetent syngeneic BALB/c mice [135]. In this study, improved efficacy by the combinatorial regimen was linked to the reconfiguration of the immunological TME in favor of preserved infiltration by $CD4^+$ cells (which was inhibited by doxorubicin alone) and persisted in a neoadjuvant model involving surgical tumor resection 6 days after treatment initiation [135]. Collectively, these studies demonstrate that (at least some) oncolytic peptides administered intratumorally can be conveniently combined with commonly used chemotherapeutics to achieve superior tumor control, not only against human cancer cell lines maintained *in vitro* or xenografted in immunodeficient mice, but also against

mouse neoplasms growing in syngeneic, immunocompetent hosts. Thus, the intratumoral administration of oncolytic peptides appears to be fully compatible with the ability of these chemotherapeutics (especially doxorubicin) to engage the host immune system in support of therapeutic efficacy [136].

Further supporting this contention, some oncolytic peptides have demonstrated promising combinatorial efficacy upon intratumoral delivery in the context of systemic immunotherapy. For instance, LL-37 has been shown to cooperate with CpG oligodeoxynucleotides, which mediate immunostimulatory effects by triggering Toll-like receptor 9 (TLR9) signaling [137], in the control of murine ovarian surface epithelial cells (MOSEC) ID8 cells growing in C57BL/6 mice [138]. Such an increased efficacy was accompanied by superior peritoneal infiltration by $F4/80^+$ macrophages and $NK1.1^+$ cells (which encompass NK cells as well as a fraction of activated $CD8^+$ CTLs) [139], and (1) was paralleled by increased expression of CD69 (an activation marker) and interferon gamma (IFNG) on the $NK1.1^+$ compartment, and (2) could be abolished by depletion of $NK1.1^+$ cells (but not macrophages) [138]. Along similar lines, EP-100 cooperated with an ICI targeting the programmed cell death 1 (PDCD1, best known as PD-1) ligand CD274 (best known as PD-L1) against mouse ID8 and IG10 ovarian cancer cells growing in immunocompetent syngeneic hosts [140], correlating with increased tumor infiltration by $CD8^+$ CTLs, NK cells and DCs and depletion of intratumoral immunosuppressive cells such as $CD4^+CD25^+FOXP3^+$ regulatory T (T_{REG}) cells [140]. Of note, IL33 secretion by cancer cells exposed to EP-100 appeared to be mechanistically relevant for the immunological reconfiguration of the TME driven by EP-100 [140].

Finally, both LTX-315 and LTX-401 administered intratumorally cooperated with systemic ICIs targeting cytotoxic T lymphocyte-associated protein 4 (CTLA4) and/or PD-1 in the control of mouse MCA205 fibrosarcomas and TC-1 lung carcinomas growing in C57BL/6 mice [99, 106]. Importantly, in both these settings, treatment schedule stood out as an important determinant of efficacy, especially with

respect to the activation of a systemic immune response that controlled contralateral lesions not receiving oncolytic peptides (so-called anenestic responses) [99, 106, 141]. Moreover, the synergistic interaction between LTX-315 and CTLA4-targeting ICIs could be reduced by blocking interleukin 2 receptor, beta chain (IL2RB, better known as CD122) [106], strongly pointing to mechanistic implication of lymphocyte-dependent adaptive immunity.

Taken together, these observations suggest that oncolytic peptides can be successfully harnessed to initiate anticancer immune responses that can be boosted by ICIs and other (immune)therapeutic agents despite ITH (**Fig. 3**), in thus far resembling other strategies that are commonly used to inflame the TME, such as RT and oncolytic virotherapy (**Box 4**). This suggests the existence of various avenues for integrating oncolytic peptides in cancer (immuno)therapy that require attentive preclinical and clinical evaluation.

Concluding remarks

Accumulating evidence indicates that oncolytic peptides constitute valuable tools to enable robust anticancer immune responses despite ITH, largely reflecting their capacity to preferentially kill malignant cells based on rather homogenous surface properties coupled to the ICD-dependent recruitment and activation of immune effector cells. However, as the clinical development of these agents for oncological indications is still in its infancy (**Table 1**), several challenges lay ahead (see **Outstanding Questions**).

First, most oncolytic peptides developed for cancer therapy so far operate by targeting lipid bilayers in cancer cells (including the plasma membrane, mitochondrial membranes, the ER membrane and the Golgi apparatus membrane) in a relatively non-specific manner [23, 30, 53, 62, 69, 82, 83]. Thus, although these agents have demonstrated consistent immunogenicity in preclinical tumor models, whether peptides with restricted activity on specific membranous compartments would mediate improved immunogenicity and/or efficacy remains to be determined. Although developing such agents may be technically complicated, protein-protein interactions may offer a convenient way to localize membrane-permeabilizing moieties in the proximity of specific subcellular compartments [142-144]. Second, whereas oncolytic peptides appear to preferentially target malignant cells based on their surface properties, the actual degree of interaction between these agents and immune cells remain to be elucidated. As discussed above, some oncolytic peptides interact directly with immune cells to stimulate their effector functions [106, 114, 116, 117, 122], not only suggesting that the mechanism of action of these agents may not be as simple as initially thought, but also raising caution on largely unexplored interactions between oncolytic peptides and immunotherapy. Third, while considerable efforts have been dedicated to optimizing the interaction between oncolytic peptides and the plasma membrane of cancer cells, limited work has been performed to engineer oncolytic peptides with added or alternative functions, such as the ability to inhibit caspases, or a delayed mode of action. Indeed, post-mitochondrial apoptotic

caspases such as CASP9, CASP3 and CASP7 have been attributed robust immunosuppressive effects in variety of settings associated with ICD induction [145-147], at least in part owing to their capacity to precipitate the terminal inactivation of dying (and hence still metabolically active) cells. Finally, the successful clinical implementation of oncolytic peptides for cancer therapy calls for the identification of potential mechanisms of resistance and strategies to circumvent them. It is known that the surface properties of cancer cells are critical for oncolytic peptides to preferentially bind and lyse their target [148], and early work suggests that first-generation oncolytic peptides (*e.g.*, LTF) are inhibited upon interaction with specific glycosaminoglycans (e.g., heparan sulfate) [40]. Although at least some next-generation oncolytic peptides appear to be minimally affected by this issue, it remains possible that other negatively charged surface molecules may interfere with their activity, standing out as potential targets for the development of combinatorial strategies with improved functionality.

Nevertheless, oncolytic peptides stand out as promising agents to target cancer cells irrespective of ITH, resulting in the initiation of a polyclonal, tumor-targeting immune response that can be further boosted with ICIs or other (immuno)therapeutic modalities. Currently explored clinical avenues include indeed the use of oncolytic peptides as therapeutics in combination with ICIs (NCT01986426) or peptide-based vaccines (NCT01223209) as well as the use of oncolytic peptides as tools to enrich the TME for tumor-infiltrating lymphocytes in preparation for adoptive cell therapy (NCT03725605). Further work is urgently needed to translate the promise of oncolytic peptide into a clinical reality.

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Table 1. Clinical development of oncolytic peptides for cancer therapy.*

Agent	Indication	Phase	Status	Notes	NCT number
CyPep-1	Advanced solid tumors	I-II	Recruiting	As standalone intratumoral agent	NCT04260529
EP-100	Advanced solid tumors	I	Completed	As standalone intravenous agent	NCT00949559
EP-100	Ovarian carcinoma	II	Completed	Optionally in combination with paclitaxel	NCT01485848
LL-37	Melanoma	I-II	Active, not recruiting	As standalone intratumoral agent	NCT02225366
LTX-315	Carcinomas	I	Completed	As adjuvant to peptide-based vaccination	NCT01223209
	Soft tissue sarcoma	II	Recruiting	In preparation for adoptive cell therapy	NCT03725605
	Transdermally accessible tumors	I	Completed	As standalone intratumoral agent	NCT01058616
				Optionally in combination with ipilimumab or pembrolizumab	NCT01986426

*as per http://www.clinicaltrials/gov, last consulted on 2020, Oct 30th

Box 1. Sources of intratumoral heterogeneity.

Most, if not all, human solid tumors display (at least some degree of) intratumoral heterogeneity (ITH), largely reflecting the co-evolution of the tumor micro-ecosystem (encompassing malignant, endothelial, stromal and immune cells) over space and time [1, 7]. For such Darwinian co-evolution to be successful and enable tumors to progress into a clinically manifest disease, recently transformed cells must be able to generate sufficient clonal diversity despite the multipronged constraints that they encounter in the tumor microenvironment (TME), and in particular (1) dwindling nutrient and oxygen levels, (2) poor availability of growth factors, and (3) active immunosurveillance [149]. However, at least in the initial phases of the disease, the genome of malignant cells is fairly similar to that of their normal counterparts, implying that genetic mutations (which require cell proliferation for being fixed in the genome) are unlikely to constitute a very early driver of ITH [150, 151]. Conversely, epigenetic alterations that enable some degree of proliferation may play a key role in the initial phases of tumor diversification by increasing the likelihood of cancer cells to acquire and fix additional mutations that offer an evolutionary advantage [4]. In this setting, a major driver of ITH is represented by mutations that interfere with (but do not fully inactivate) the molecular machinery for DNA repair, ultimately enabling considerable degrees of genetic/genomic instability and accelerated tumor progression [152]. This is paralleled by the generation of a highly diverse population of malignant cells that represent a perfect evolutionary substrate for the survival of tumor despite microenvironmental conditions that change over space – as in different (micro)anatomical locations – and time – as in response to treatment [7]. As a corollary of this model, recently transformed malignant cells that are unable to generate sufficient diversity at early stages of the disease are expected to be susceptible to changing microenvironmental conditions. It has also been proposed that only a minority of neoplastic cell precursors that originate over a lifetime ultimately form

progressing tumors that manifest clinically, while the vast majority of them die, are unable to proliferate or are eliminated by cancer immunosurveillance [153, 154].

Box 2. History of oncolysis with peptides.

1922. Discovery of the first natural peptide with bacteriolytic activity, lysozyme [155].

1963. Identification of bactericidal basic proteins in the lysosomal fraction of human neutrophils [156].

1985. Isolation of defensins from human neutrophils [157].

1986. First demonstration that human and rabbit defensins mediate cytolytic activity against cancer cells [158].

1991. Cloning of CAMP from rabbit leukocytes [159].

2007. First report on the ability of a CAMP-derived peptide (LL-37) to boost TLR9 signaling in plasmacytoid dendritic cells [114].

2009. Initial demonstration that LL-37 synergizes with CpG-based immunotherapy in immunocompetent tumor models [138].

2009. First clinical trial investigating the pharmacodynamics and pharmacokinetics of a GNRHR-targeted peptide (EP-100) in patients with solid tumors (NCT00949559).

2013. First clinical study investigating safety, tolerability, pharmacokinetics and efficacy of a lactotransferrin-inspired synthetic peptide (LTX-315) combined with ICIs in patients with transdermally accessible tumors (NCT01986426).

2014. First formal demonstration that LTX-315 drives *bona fide* immunogenic cell death [51].

2014. First safety report from a clinical trial testing oncolysis with EP-100 in cancer patients (NCT01485848) [160].

2015. First study on the capacity of the AMP nisin Z to synergize with immunogenic chemotherapy in immunocompetent tumor models [134].

2016. First demonstration of the ability of LTX-315 to synergize with ICIs in immunocompetent tumor models [106].

2019. Case report documenting immune infiltration and clinical activity in patients with desmoid sarcoma receiving LTX-315 *i.t.* [102].

Box 3. Common structural features of oncolytic peptides.

Most modern oncolytic peptides have been developed by optimizing the biochemical and structural properties of natural antimicrobial peptides (AMPs), which are relatively short (12-50 aa, in animals) polypeptides synthesized by a large variety of organisms (including mammals, plants, lower eukaryotes, and prokaryotes) as an innate defense against viral, bacterial and fungal pathogens or competing species [161, 162]. As a very large protein family (more than 2,600 members have been characterized so far) [163], AMPs do not appear to share conserved functional domains, but are generally characterized by a high proportion of positively charged (*i.e.*, Arg, Lys) and hydrophobic (i.e., Ala, Val, Gly), residues, often conferring a global amphipathic nature to the molecule. These features endow AMPs with the capacity to bind in a non-specific manner negatively charged phospholipids that are abundant on the surface of microbes, such as phosphatidylglycerol [162], or cancer cells, such as phosphatidylserine (normally secluded in the inner leaflet of the plasma membrane in normal cells) [164]. Natural AMPs exist in five different structural conformations (α-helical, β-sheet, mixed, cyclic or unstructured), and while many of these molecules (especially when of mammalian origin) exhibit at least some degree of specificity for microbial or cancer cell membranes, such as human lysozyme (LYZ) and cathelicidin antimicrobial peptide (CAMP), some AMPs exhibit limited selectively, including various AMPs found in the bee venom [165, 166]. Importantly, while some AMPs such as LYZ *de facto* exert their activity at the plasma membrane by eliciting a direct cytolytic effect (that may also involve intracellular membranes) [167], others may operate irrespective of direct cytolysis via either extracellular or intracellular mechanisms, such as lactotransferrin (LTF) [168] and histatin 3 (HTN3) fragments [169], respectively. Oncolytic peptides that have been investigated for their anticancer properties encompass a variety of structural configurations, including full-length AMPs, such as defensin alpha 1 (DEFA1)

[170], AMP-derived moieties, such as the C-terminus of CAMP (LL-37) [171], as well as synthetic peptides designed on structure-activity relationship studies of natural AMPs, such as LTX-315 [53, 172].

Box 4. Non-peptide strategies to inflame the tumor microenvironment.

Neoplastic lesions that exhibit poor infiltration by immune effector cells (so-called "cold tumors") are generally resistant to a variety of treatments including (but not limited to) immune checkpoint inhibitors (ICIs), and hence are often associated with poor disease outcome [173]. Thus, considerable efforts have been dedicated to the identification of agents that would "inflame" cold tumors and convert them into highly infiltrated (so-called "hot") lesions [174], which instead are relatively amenable to (immuno)therapeutic interventions [173]. While multiple oncolytic peptides have been shown to favor tumor infiltration by effector cells that support anticancer immunity including (but not limited to) dendritic cell (DC) precursors and $CD8^+$ cytotoxic T lymphocytes (see Main text), several other therapeutic strategies may be harnessed to inflame the tumor microenvironment (TME) in favor of responsiveness to (immuno)therapy [175]. These approaches include (but are not limited to):

- induction of immunogenic cell death (ICD) with (1) selected chemotherapeutics, such as anthracyclines (e.g., doxorubicin, mitoxantrone) [136] and platinum-containing agents (e.g., oxaliplatin, PT-112) [176, 177]; (2) oncolytic virotherapy [178]; (3) radiation therapy [179-181]; and (4) photodynamic therapy (PDT) [175]

- intratumoral administration of immunostimulatory agents that initiate pattern recognition receptor (PRR) signaling, such as (1) stimulator of interferon response cGAMP interactor 1 (STING1) agonists [182, 183]; (2) Toll-like receptor (TLR) activators [137, 184]; (3) DExD/H-box helicase 58 (DDX58, best known as RIG-I) [185] and (4) inactivated/weakened bacterial or viral preparations, such as the so-called Bacillus Calmette-Guérin (BCG) [186] and rotavirus vaccines [187]

- intratumoral administration or expression of (1) recombinant immunostimulatory cytokines such as interleukin 12 (IL12) [188]; (2) ICIs such as the cytotoxic T lymphocyte-activated protein (CTLA4)-

targeting agent ipilimumab [189]; and (3) other immunostimulatory agents, including antibodies that promote co-activatory signaling in T cells [190].

In this context, directly targeting the TME with intratumoral approaches offers a number of advantages over systemic strategies. Specifically, intratumoral administration enables the use of molecules that may be poorly tolerated on systemic delivery, and contains the amount of drugs required to achieve therapeutic doses (which is especially important for expensive agents such as ICIs), standing out as a particularly promising approach for inflaming the TME [141, 191, 192].

Legends to Display Items

Figure 1. **Main cellular targets for oncolytic peptides.** While the mechanisms of action of antimicrobial peptides exhibit considerable variability, most oncolytic peptides currently in preclinical and clinical development for cancer therapy primarily operate by targeting membranous compartments, including the plasma membrane, mitochondria, the endoplasmic reticulum (ER) and the Golgi apparatus (GA). Thus, the ultimate mechanism through which oncolytic peptides mediate cytotoxic effects against a specific cellular target depend at least on two parameters: (1) the relative affinity of the peptide for specific cellular membranes, and (2) the overall configuration of the signaling network that precipitate regulated cell death (RCD). This explains why instances of apoptosis, mitochondrial permeability transition (MPT)-driven regulated necrosis, necroptosis as well as unregulated necrosis in the context of accidental cell death (ACD) have been reported in cancer cells exposed to oncolytic peptides.

Figure 2. **Immunological effects of oncolytic peptides.** Several oncolytic peptides have been shown to mediate immunostimulatory effects by eliciting an immunogenic variant of regulated cell death that is associated with the abundant emission of antigens and danger signals from dying cells. This enables the recruitment and activation of antigen presenting cells (APCs), which – upon engulfment of antigenic material from dying cancer cells – migrate to lymph nodes or tertiary lymphoid structures and prime tumor-specific T cells. Along with a reconfiguration of the tumor microenvironment (TME) towards an immunostimulatory profile, the influx of $CD8^+$ cytotoxic T lymphocytes (CTLs) favors local disease control and (at least in some settings) activation of robust anticancer immunity with systemic outreach and coupled to the establishment of immunological memory.

Figure 3. **Oncolytic peptides to target cancer heterogeneity.** Oncolytic peptides stand out as promising agents to overcome (at least some degree of) intratumoral heterogeneity (ITH), largely reflecting (1) their ability to target cancer cells based on rather homogeneous cell surface properties, and (2) their capacity

to drive immunogenic cell death in the context of an abundant release of antigenic material. An expanding preclinical literature indicates that oncolytic peptides can be conveniently combined with numerous commonly employed and experimental therapeutics to achieve superior disease control despite ITH. ICI, immune checkpoint inhibitor; TLR9, Toll-like receptor 9.

Glossary. Cell-death related terminology.

Accidental cell death (ACD). Variant of cell death that is initiated by harsh microenvironmental conditions and largely reflects the physical disassembly/irreversible permeabilization of cellular membranes.

Apoptosis. Instance of cell death that – irrespective of initiation by extracellular or intracellular stress – is precipitated by the activation of proteolytic enzymes from the caspase family.

Immunogenic cell death (ICD). Form of cell death that – in immunocompetent syngeneic hosts – is sufficient to initiate an adaptive immune response against dead cell-associated antigens.

Mitochondrial membrane permeabilization (MOMP). BCL2 apoptosis regulator (BCL2)-inhibitable loss of selective permeability at the outer mitochondrial membrane that culminates with the release of caspase activators and other cytotoxic proteins in the cytosol.

Mitochondrial permeability transition (MPT). Peptidylprolyl isomerase F (PPIF)-dependent rapid loss of selective permeability at the inner mitochondrial membrane, resulting in immediate abrogation of respiratory capacity, mitochondrial swelling and cell death via caspase-independent mechanisms.

Necroptosis. Regulated form of necrosis that mechanistically involves receptor interacting serine/threonine kinase 3 (RIPK3)-initiated, mixed lineage kinase domain like pseudokinase (MLKL)-dependent plasma membrane permeabilization.

Regulated cell death (RCD). Variant of cell death that occurs in the context of failing adaptation to changing microenvironmental conditions and involves the activation of genetically-encoded dedicated molecular mechanisms.

References

1. McGranahan, N. and Swanton, C. (2017) Clonal Heterogeneity and Tumor Evolution: Past, Present, and the Future. Cell 168 (4), 613-628.

2. Nowell, P.C. (1976) The clonal evolution of tumor cell populations. Science 194 (4260), 23-8.

3. Jamal-Hanjani, M. et al. (2017) Tracking the Evolution of Non-Small-Cell Lung Cancer. N Engl J Med 376 (22), 2109-2121.

4. Teixeira, V.H. et al. (2019) Deciphering the genomic, epigenomic, and transcriptomic landscapes of pre-invasive lung cancer lesions. Nat Med 25 (3), 517-525.

5. Biswas, D. et al. (2019) A clonal expression biomarker associates with lung cancer mortality. Nat Med 25 (10), 1540-1548.

6. Hensley, C.T. et al. (2016) Metabolic Heterogeneity in Human Lung Tumors. Cell 164 (4), 681-94.

7. Dagogo-Jack, I. and Shaw, A.T. (2018) Tumour heterogeneity and resistance to cancer therapies. Nat Rev Clin Oncol 15 (2), 81-94.

8. Salmon, H. et al. (2019) Host tissue determinants of tumour immunity. Nat Rev Cancer 19 (4), 215-227.

9. Birkbak, N.J. and McGranahan, N. (2020) Cancer Genome Evolutionary Trajectories in Metastasis. Cancer Cell 37 (1), 8-19.

10. Marusyk, A. et al. (2020) Intratumor Heterogeneity: The Rosetta Stone of Therapy Resistance. Cancer Cell 37 (4), 471-484.

11. Li, S. et al. (2016) Distinct evolution and dynamics of epigenetic and genetic heterogeneity in acute myeloid leukemia. Nat Med 22 (7), 792-9.

12. Lin, D.C. et al. (2017) Genomic and Epigenomic Heterogeneity of Hepatocellular Carcinoma. Cancer Res 77 (9), 2255-2265.

13. Schumacher, T.N. et al. (2019) Cancer Neoantigens. Annu Rev Immunol 37, 173-200.

14. Le, D.T. et al. (2015) PD-1 Blockade in Tumors with Mismatch-Repair Deficiency. N Engl J Med 372 (26), 2509-20.

15. Galluzzi, L. et al. (2018) The hallmarks of successful anticancer immunotherapy. Sci Transl Med 10 (459).

16. Sansregret, L. et al. (2018) Determinants and clinical implications of chromosomal instability in cancer. Nat Rev Clin Oncol 15 (3), 139-150.

17. Andor, N. et al. (2017) Genomic Instability in Cancer: Teetering on the Limit of Tolerance. Cancer Res 77 (9), 2179-2185.

18. Tang, Y.C. et al. (2011) Identification of aneuploidy-selective antiproliferation compounds. Cell 144 (4), 499-512.

19. Janssen, A. et al. (2009) Elevating the frequency of chromosome mis-segregation as a strategy to kill tumor cells. Proc Natl Acad Sci U S A 106 (45), 19108-13.

20. Sansregret, L. et al. (2017) APC/C Dysfunction Limits Excessive Cancer Chromosomal Instability. Cancer Discov 7 (2), 218-233.

21. Yang, N. et al. (2002) Enhanced antitumor activity and selectivity of lactoferrin-derived peptides. J Pept Res 60 (4), 187-97.

22. Torres, M.D.T. et al. (2020) The wasp venom antimicrobial peptide polybia-CP and its synthetic derivatives display antiplasmodial and anticancer properties. Bioeng Transl Med 5 (3), e10167.

23. Li, C. et al. (2018) N-myristoylation of Antimicrobial Peptide CM4 Enhances Its Anticancer Activity by Interacting With Cell Membrane and Targeting Mitochondria in Breast Cancer Cells. Front Pharmacol 9, 1297.

24. Rekdal, Ø. et al. (2012) Relative spatial positions of tryptophan and cationic residues in helical membrane-active peptides determine their cytotoxicity. J Biol Chem 287 (1), 233-44.

25. Gaspar, D. et al. (2012) Anticancer peptide SVS-1: efficacy precedes membrane neutralization. Biochemistry 51 (32), 6263-5.

26. Eliassen, L.T. et al. (2003) Enhanced antitumour activity of 15-residue bovine lactoferricin derivatives containing bulky aromatic amino acids and lipophilic N-terminal modifications. J Pept Sci 9 (8), 510-7.

27. Liu, X. et al. (2016) Amphipathicity Determines Different Cytotoxic Mechanisms of Lysine- or Arginine-Rich Cationic Hydrophobic Peptides in Cancer Cells. J Med Chem 59 (11), 5238-47.

28. Zhang, W. et al. (2010) A novel analog of antimicrobial peptide Polybia-MPI, with thioamide bond substitution, exhibits increased therapeutic efficacy against cancer and diminished toxicity in mice. Peptides 31 (10), 1832-8.

29. Utsugi, T. et al. (1991) Elevated expression of phosphatidylserine in the outer membrane leaflet of human tumor cells and recognition by activated human blood monocytes. Cancer Res 51 (11), 3062-6.

30. Wodlej, C. et al. (2019) Interaction of two antitumor peptides with membrane lipids - Influence of phosphatidylserine and cholesterol on specificity for melanoma cells. PLoS One 14 (1), e0211187.

31. Riedl, S. et al. (2015) Human lactoferricin derived di-peptides deploying loop structures induce apoptosis specifically in cancer cells through targeting membranous phosphatidylserine. Biochim Biophys Acta 1848 (11 Pt A), 2918-31.

32. Riedl, S. et al. (2014) Killing of melanoma cells and their metastases by human lactoferricin derivatives requires interaction with the cancer marker phosphatidylserine. Biometals 27 (5), 981-97.

33. Baxter, A.A. et al. (2017) The plant defensin NaD1 induces tumor cell death via a non-apoptotic, membranolytic process. Cell Death Discov 3, 16102.

34. Phan, T.K. et al. (2016) Human β-defensin 3 contains an oncolytic motif that binds PI(4,5)P2 to mediate tumour cell permeabilisation. Oncotarget 7 (2), 2054-69.

35. Baxter, A.A. et al. (2015) The Tomato Defensin TPP3 Binds Phosphatidylinositol (4,5)-Bisphosphate via a Conserved Dimeric Cationic Grip Conformation To Mediate Cell Lysis. Mol Cell Biol 35 (11), 1964-78.

36. Poon, I. et al. (2014) Phosphoinositide-mediated oligomerization of a defensin induces cell lysis. Elife 3, e01808.

37. Dos Santos, C. et al. (2017) Studies of the antitumor mechanism of action of dermaseptin B2, a multifunctional cationic antimicrobial peptide, reveal a partial implication of cell surface glycosaminoglycans. PLoS One 12 (8), e0182926.

38. Lee, H.S. et al. (2008) Mechanism of anticancer activity of buforin IIb, a histone H2A-derived peptide. Cancer Lett 271 (1), 47-55.

39. Chen, Y.Q. et al. (2010) A cationic amphiphilic peptide ABP-CM4 exhibits selective cytotoxicity against leukemia cells. Peptides 31 (8), 1504-10.

40. Fadnes, B. et al. (2009) The anticancer activity of lytic peptides is inhibited by heparan sulfate on the surface of the tumor cells. BMC Cancer 9, 183.

41. Fadnes, B. et al. (2011) Small lytic peptides escape the inhibitory effect of heparan sulfate on the surface of cancer cells. BMC Cancer 11, 116.

42. Crusca, E., Jr. et al. (2018) Biophysical characterization and antitumor activity of synthetic Pantinin peptides from scorpion's venom. Biochim Biophys Acta Biomembr 1860 (11), 2155-2165.

43. Banković, J. et al. (2013) The elimination of P-glycoprotein over-expressing cancer cells by antimicrobial cationic peptide NK-2: the unique way of multi-drug resistance modulation. Exp Cell Res 319 (7), 1013-27.

44. Makovitzki, A. et al. (2009) Suppression of human solid tumor growth in mice by intratumor and systemic inoculation of histidine-rich and pH-dependent host defense-like lytic peptides. Cancer Res 69 (8), 3458-63.

45. Zhao, H. et al. (2017) The development of activatable lytic peptides for targeting triple negative breast cancer. Cell Death Discov 3, 17037.

46. Galluzzi, L. et al. (2018) Molecular mechanisms of cell death: recommendations of the Nomenclature Committee on Cell Death 2018. Cell Death Differ 25 (3), 486-541.

47. da Silva, A.M.B. et al. (2018) Pro-necrotic Activity of Cationic Mastoparan Peptides in Human Glioblastoma Multiforme Cells Via Membranolytic Action. Mol Neurobiol 55 (7), 5490-5504.

48. Solarte, V.A. et al. (2015) A Tetrameric Peptide Derived from Bovine Lactoferricin Exhibits Specific Cytotoxic Effects against Oral Squamous-Cell Carcinoma Cell Lines. Biomed Res Int 2015, 630179.

49. Chen, Y.C. et al. (2015) Anti-proliferative effect on a colon adenocarcinoma cell line exerted by a membrane disrupting antimicrobial peptide KL15. Cancer Biol Ther 16 (8), 1172-83.

50. Chen, J.Y. et al. (2009) A fish antimicrobial peptide, tilapia hepcidin TH2-3, shows potent antitumor activity against human fibrosarcoma cells. Peptides 30 (9), 1636-42.

51. Camilio, K.A. et al. (2014) Complete regression and systemic protective immune responses obtained in B16 melanomas after treatment with LTX-315. Cancer Immunol Immunother 63 (6), 601-13.

52. Forveille, S. et al. (2015) The oncolytic peptide LTX-315 triggers necrotic cell death. Cell Cycle 14 (21), 3506-12.

53. Haug, B.E. et al. (2016) Discovery of a 9-mer Cationic Peptide (LTX-315) as a Potential First in Class Oncolytic Peptide. J Med Chem 59 (7), 2918-27.

54. Lehmann, J. et al. (2006) Antitumor activity of the antimicrobial peptide magainin II against bladder cancer cell lines. Eur Urol 50 (1), 141-7.

55. Tian, Y. et al. (2013) The cathelicidin-BF Lys16 mutant Cbf-K16 selectively inhibits non-small cell lung cancer proliferation in vitro. Oncol Rep 30 (5), 2502-10.

56. Wang, H. et al. (2013) BF-30 selectively inhibits melanoma cell proliferation via cytoplasmic membrane permeabilization and DNA-binding in vitro and in B16F10-bearing mice. Eur J Pharmacol 707 (1-3), 1-10.

57. Steinstraesser, L. et al. (2011) Oncolytic designer host defense peptide suppresses growth of human liposarcoma. Int J Cancer 128 (12), 2994-3004.

58. Steinstraesser, L. et al. (2011) Suppression of soft tissue sarcoma growth by a host defense-like lytic peptide. PLoS One 6 (3), e18321.

59. Papo, N. et al. (2006) Inhibition of tumor growth and elimination of multiple metastases in human prostate and breast xenografts by systemic inoculation of a host defense-like lytic peptide. Cancer Res 66 (10), 5371-8.

60. Szczepanski, C. et al. (2014) Identification of a novel lytic peptide for the treatment of solid tumours. Genes Cancer 5 (5-6), 186-200.

61. Papo, N. et al. (2004) Suppression of human prostate tumor growth in mice by a cytolytic D-, L-amino Acid Peptide: membrane lysis, increased necrosis, and inhibition of prostate-specific antigen secretion. Cancer Res 64 (16), 5779-86.

62. Zhou, H. et al. (2015) The oncolytic peptide LTX-315 kills cancer cells through Bax/Bak-regulated mitochondrial membrane permeabilization. Oncotarget 6 (29), 26599-614.

63. Eliassen, L.T. et al. (2006) The antimicrobial peptide, lactoferricin B, is cytotoxic to neuroblastoma cells in vitro and inhibits xenograft growth in vivo. Int J Cancer 119 (3), 493-500.

64. Insuasty-Cepeda, D.S. et al. (2020) Peptides Derived from (RRWQWRMKKLG)2-K-Ahx Induce Selective Cellular Death in Breast Cancer Cell Lines through Apoptotic Pathway. Int J Mol Sci 21 (12).

65. Lewies, A. et al. (2018) The antimicrobial peptide nisin Z induces selective toxicity and apoptotic cell death in cultured melanoma cells. Biochimie 144, 28-40.

66. Xu, P. et al. (2020) Inhibitory effects of Bombyx mori antimicrobial peptide cecropins on esophageal cancer cells. Eur J Pharmacol 887, 173434.

67. Wu, Y.L. et al. (2015) CecropinXJ inhibits the proliferation of human gastric cancer BGC823 cells and induces cell death in vitro and in vivo. Int J Oncol 46 (5), 2181-93.

68. Cerón, J.M. et al. (2010) The antimicrobial peptide cecropin A induces caspase-independent cell death in human promyelocytic leukemia cells. Peptides 31 (8), 1494-503.

69. Su, B.C. et al. (2020) Antimicrobial Peptide TP4 Targets Mitochondrial Adenine Nucleotide Translocator 2. Mar Drugs 18 (8).

70. Chen, Y.F. et al. (2020) TP3, an antimicrobial peptide, inhibits infiltration and motility of glioblastoma cells via modulating the tumor microenvironment. Cancer Med 9 (11), 3918-3931.

71. Glab, J.A. et al. (2020) Bcl-2 family proteins, beyond the veil. Int Rev Cell Mol Biol 351, 1-22.

72. Mader, J.S. et al. (2005) Bovine lactoferricin selectively induces apoptosis in human leukemia and carcinoma cell lines. Mol Cancer Ther 4 (4), 612-24.

73. Mader, J.S. et al. (2009) The human host defense peptide LL-37 induces apoptosis in a calpain- and apoptosis-inducing factor-dependent manner involving Bax activity. Mol Cancer Res 7 (5), 689-702.

74. Okumura, K. et al. (2004) C-terminal domain of human CAP18 antimicrobial peptide induces apoptosis in oral squamous cell carcinoma SAS-H1 cells. Cancer Lett 212 (2), 185-94.

75. Ren, S.X. et al. (2012) Host immune defense peptide LL-37 activates caspase-independent apoptosis and suppresses colon cancer. Cancer Res 72 (24), 6512-23.

76. Kuo, H.M. et al. (2018) MSP-4, an Antimicrobial Peptide, Induces Apoptosis via Activation of Extrinsic Fas/FasL- and Intrinsic Mitochondria-Mediated Pathways in One Osteosarcoma Cell Line. Mar Drugs 16 (1).

77. Dong, Z. et al. (2020) Novel Frog Skin-Derived Peptide Dermaseptin-PP for Lung Cancer Treatment: In vitro/vivo Evaluation and Anti-tumor Mechanisms Study. Front Chem 8, 476.

78. Risso, A. et al. (2002) BMAP-28, an antibiotic peptide of innate immunity, induces cell death through opening of the mitochondrial permeability transition pore. Mol Cell Biol 22 (6), 1926-35.

79. Zhou, H. et al. (2018) Oncolysis with DTT-205 and DTT-304 generates immunological memory in cured animals. Cell Death Dis 9 (11), 1086.

80. Su, B.C. et al. (2019) Antimicrobial Peptide TP4 Induces ROS-Mediated Necrosis by Triggering Mitochondrial Dysfunction in Wild-Type and Mutant p53 Glioblastoma Cells. Cancers (Basel) 11 (2).

81. Lin, W.J. et al. (2009) Epinecidin-1, an antimicrobial peptide from fish (Epinephelus coioides) which has an antitumor effect like lytic peptides in human fibrosarcoma cells. Peptides 30 (2), 283-90.

82. Lv, Y. et al. (2019) The antimicrobial peptide PFR induces necroptosis mediated by ER stress and elevated cytoplasmic calcium and mitochondrial ROS levels: cooperation with Ara-C to act against acute myeloid leukemia. Signal Transduct Target Ther 4, 38.

83. Zhou, H. et al. (2016) The oncolytic compound LTX-401 targets the Golgi apparatus. Cell Death Differ 23 (12), 2031-2041.

84. Gomes-da-Silva, L.C. et al. (2019) Recruitment of LC3 to damaged Golgi apparatus. Cell Death Differ 26 (8), 1467-1484.

85. Ghavami, S. et al. (2008) Brevinin-2R(1) semi-selectively kills cancer cells by a distinct mechanism, which involves the lysosomal-mitochondrial death pathway. J Cell Mol Med 12 (3), 1005-22.

86. Ting, C.H. et al. (2016) Targeting FOSB with a cationic antimicrobial peptide, TP4, for treatment of triple-negative breast cancer. Oncotarget 7 (26), 40329-40347.

87. Wang, J. et al. (2019) Cathelicidin Suppresses Colon Cancer Metastasis via a P2RX7-Dependent Mechanism. Mol Ther Oncolytics 12, 195-203.

88. Li, B. et al. (2018) Triggering of cancer cell cycle arrest by a novel scorpion venom-derived peptide-Gonearrestide. J Cell Mol Med 22 (9), 4460-4473.

89. Kuroda, K. et al. (2015) Antimicrobial peptide FF/CAP18 induces apoptotic cell death in HCT116 colon cancer cells via changes in the metabolic profile. Int J Oncol 46 (4), 1516-26.

90. Galluzzi, L. et al. (2020) Consensus guidelines for the definition, detection and interpretation of immunogenic cell death. J Immunother Cancer 8 (1).

91. Chen, D.S. and Mellman, I. (2017) Elements of cancer immunity and the cancer-immune set point. Nature 541 (7637), 321-330.

92. Martinek, J. et al. (2019) Interplay between dendritic cells and cancer cells. Int Rev Cell Mol Biol 348, 179-215.

93. Kotsias, F. et al. (2019) Antigen processing and presentation. Int Rev Cell Mol Biol 348, 69-121.

94. Sprooten, J. et al. (2019) Type I interferons and dendritic cells in cancer immunotherapy. Int Rev Cell Mol Biol 348, 217-262.

95. Eike, L.M. et al. (2015) The oncolytic peptide LTX-315 induces cell death and DAMP release by mitochondria distortion in human melanoma cells. Oncotarget 6 (33), 34910-23.

96. Zhou, H. et al. (2016) The oncolytic peptide LTX-315 triggers immunogenic cell death. Cell Death Dis 7 (3), e2134.

97. Eike, L.M. et al. (2016) The Cytolytic Amphipathic β(2,2)-Amino Acid LTX-401 Induces DAMP Release in Melanoma Cells and Causes Complete Regression of B16 Melanoma. PLoS One 11 (2), e0148980.

98. Pasquereau-Kotula, E. et al. (2018) The anticancer peptide RT53 induces immunogenic cell death. PLoS One 13 (8), e0201220.

99. Xie, W. et al. (2019) Tumor lysis with LTX-401 creates anticancer immunity. Oncoimmunology 8 (7), 1594555.

100. Wang, Y.S. et al. (2009) Intratumoral expression of mature human neutrophil peptide-1 mediates antitumor immunity in mice. Clin Cancer Res 15 (22), 6901-11.

101. Liao, H.W. et al. (2019) LTX-315 sequentially promotes lymphocyte-independent and lymphocyte-dependent antitumor effects. Cell Stress 3 (11), 348-360.

102. Jebsen, N.L. et al. (2019) Enhanced T-lymphocyte infiltration in a desmoid tumor of the thoracic wall in a young woman treated with intratumoral injections of the oncolytic peptide LTX-315: a case report. J Med Case Rep 13 (1), 177.

103. Nestvold, J. et al. (2017) Oncolytic peptide LTX-315 induces an immune-mediated abscopal effect in a rat sarcoma model. Oncoimmunology 6 (8), e1338236.

104. Mauseth, B. et al. (2019) The Novel Oncolytic Compound LTX-401 Induces Antitumor Immune Responses in Experimental Hepatocellular Carcinoma. Mol Ther Oncolytics 14, 139-148.

105. Berge, G. et al. (2010) Therapeutic vaccination against a murine lymphoma by intratumoral injection of a cationic anticancer peptide. Cancer Immunol Immunother 59 (8), 1285-94.

106. Yamazaki, T. et al. (2016) The oncolytic peptide LTX-315 overcomes resistance of cancers to immunotherapy with CTLA4 checkpoint blockade. Cell Death Differ 23 (6), 1004-15.

107. Togashi, Y. et al. (2019) Regulatory T cells in cancer immunosuppression - implications for anticancer therapy. Nat Rev Clin Oncol 16 (6), 356-371.

108. Talmadge, J.E. and Gabrilovich, D.I. (2013) History of myeloid-derived suppressor cells. Nat Rev Cancer 13 (10), 739-52.

109. Mader, J.S. et al. (2011) The human cathelicidin, LL-37, induces granzyme-mediated apoptosis in regulatory T cells. J Immunother 34 (3), 229-35.

110. Han, Y. et al. (2015) In Vitro and in Vivo Anticancer Activity of Pardaxin against Proliferation and Growth of Oral Squamous Cell Carcinoma. Mar Drugs 14 (1), 2.

111. Ji, S.Y. et al. (2020) A Novel Peptide Oligomer of Bacitracin Induces M1 Macrophage Polarization by Facilitating Ca(2+) Influx. Nutrients 12 (6).

112. Chamilos, G. et al. (2012) Cytosolic sensing of extracellular self-DNA transported into monocytes by the antimicrobial peptide LL37. Blood 120 (18), 3699-707.

113. Ganguly, D. et al. (2009) Self-RNA-antimicrobial peptide complexes activate human dendritic cells through TLR7 and TLR8. J Exp Med 206 (9), 1983-94.

114. Lande, R. et al. (2007) Plasmacytoid dendritic cells sense self-DNA coupled with antimicrobial peptide. Nature 449 (7162), 564-9.

115. Huntington, N.D. et al. (2020) The cancer-natural killer cell immunity cycle. Nat Rev Cancer 20 (8), 437-454.

116. Kurosaka, K. et al. (2005) Mouse cathelin-related antimicrobial peptide chemoattracts leukocytes using formyl peptide receptor-like 1/mouse formyl peptide receptor-like 2 as the receptor and acts as an immune adjuvant. J Immunol 174 (10), 6257-65.

117. Cheng, M. et al. (2015) Cathelicidin suppresses colon cancer development by inhibition of cancer associated fibroblasts. Clin Exp Gastroenterol 8, 13-29.

118. Lee, J. et al. (2020) Cathelicidin-Related Antimicrobial Peptide Regulates CD73 Expression in Mouse Th17 Cells via p38. Cells 9 (6).

119. Cha, H.R. et al. (2016) Prostate cancer-derived cathelicidin-related antimicrobial peptide facilitates macrophage differentiation and polarization of immature myeloid progenitors to protumorigenic macrophages. Prostate 76 (7), 624-36.

120. Mader, J.S. et al. (2011) The human cathelicidin, LL-37, induces granzyme-mediated apoptosis in cytotoxic T lymphocytes. Exp Cell Res 317 (4), 531-8.

121. Jin, G. et al. (2010) An antimicrobial peptide regulates tumor-associated macrophage trafficking via the chemokine receptor CCR2, a model for tumorigenesis. PLoS One 5 (6), e10993.

122. Lioi, A.B. et al. (2012) Membrane damage and repair in primary monocytes exposed to human β-defensin-3. J Leukoc Biol 92 (5), 1083-91.

123. Li, D. et al. (2014) Gene therapy with beta-defensin 2 induces antitumor immunity and enhances local antitumor effects. Hum Gene Ther 25 (1), 63-72.

124. Lioi, A.B. et al. (2015) Human β Defensin-3 Increases CD86 Expression on Monocytes by Activating the ATP-Gated Channel P2X7. J Immunol 195 (9), 4438-45.

125. Conlon, J.M. et al. (2008) Characterization of antimicrobial peptides from the skin secretions of the Malaysian frogs, Odorrana hosii and Hylarana picturata (Anura:Ranidae). Toxicon 52 (3), 465-73.

126. Luo, X. et al. (2020) Antimicrobial Peptide Reverses ABCB1-Mediated Chemotherapeutic Drug Resistance. Front Pharmacol 11, 1208.

127. Barua, S. et al. (2010) Lytic peptide-mediated sensitization of TRAIL-resistant prostate cancer cells to death receptor agonists. Cancer Lett 293 (2), 240-53.

128. Ke, M. et al. (2018) MEL-pep, an analog of melittin, disrupts cell membranes and reverses 5-fluorouracil resistance in human hepatocellular carcinoma cells. Int J Biochem Cell Biol 101, 39-48.

129. Hilchie, A.L. et al. (2011) Pleurocidin-family cationic antimicrobial peptides are cytolytic for breast carcinoma cells and prevent growth of tumor xenografts. Breast Cancer Res 13 (5), R102.

130. Ting, C.H. and Chen, J.Y. (2018) Nile Tilapia Derived TP4 Shows Broad Cytotoxicity Toward to Non-Small-Cell Lung Cancer Cells. Mar Drugs 16 (12).

131. Ma, S. et al. (2019) GnRH-R-Targeted Lytic Peptide Sensitizes BRCA Wild-type Ovarian Cancer to PARP Inhibition. Mol Cancer Ther 18 (5), 969-979.

132. Hilchie, A.L. et al. (2016) Mastoparan is a membranolytic anti-cancer peptide that works synergistically with gemcitabine in a mouse model of mammary carcinoma. Biochim Biophys Acta 1858 (12), 3195-3204.

133. Rana, K. et al. (2019) Augmented therapeutic efficacy of 5-fluorouracil in conjunction with lantibiotic nisin against skin cancer. Biochem Biophys Res Commun 520 (3), 551-559.

134. Preet, S. et al. (2015) Effect of nisin and doxorubicin on DMBA-induced skin carcinogenesis--a possible adjunct therapy. Tumour Biol 36 (11), 8301-8.

135. Camilio, K.A. et al. (2019) Combining the oncolytic peptide LTX-315 with doxorubicin demonstrates therapeutic potential in a triple-negative breast cancer model. Breast Cancer Res 21 (1), 9.

136. Galluzzi, L. et al. (2020) Immunostimulation with chemotherapy in the era of immune checkpoint inhibitors. Nat Rev Clin Oncol 17 (12), 725-741.

137. Vanpouille-Box, C. et al. (2019) Pharmacological modulation of nucleic acid sensors - therapeutic potential and persisting obstacles. Nat Rev Drug Discov 18 (11), 845-867.

138. Chuang, C.M. et al. (2009) Treatment with LL-37 peptide enhances antitumor effects induced by CpG oligodeoxynucleotides against ovarian cancer. Hum Gene Ther 20 (4), 303-13.

139. Assarsson, E. et al. (2000) CD8+ T cells rapidly acquire NK1.1 and NK cell-associated molecules upon stimulation in vitro and in vivo. J Immunol 165 (7), 3673-9.

140. Kim, M.S. et al. (2020) Enhanced immunotherapy with LHRH-R targeted lytic peptide in ovarian cancer. Mol Cancer Ther.

141. Marabelle, A. et al. (2018) Starting the fight in the tumor: expert recommendations for the development of human intratumoral immunotherapy (HIT-IT). Ann Oncol 29 (11), 2163-2174.

142. Cerrato, C.P. et al. (2017) Cell-penetrating peptides with intracellular organelle targeting. Expert Opin Drug Deliv 14 (2), 245-255.

143. Maso, K. et al. (2019) Molecular platforms for targeted drug delivery. Int Rev Cell Mol Biol 346, 1-50.

144. Field, L.D. et al. (2015) Peptides for specifically targeting nanoparticles to cellular organelles: quo vadis? Acc Chem Res 48 (5), 1380-90.

145. Han, C. et al. (2020) Tumor cells suppress radiation-induced immunity by hijacking caspase 9 signaling. Nat Immunol 21 (5), 546-554.

146. Rodriguez-Ruiz, M.E. et al. (2019) Apoptotic caspases inhibit abscopal responses to radiation and identify a new prognostic biomarker for breast cancer patients. Oncoimmunology 8 (11), e1655964.

147. White, M.J. et al. (2014) Apoptotic caspases suppress mtDNA-induced STING-mediated type I IFN production. Cell 159 (7), 1549-62.

148. Baxter, A.A. et al. (2017) Tumor cell membrane-targeting cationic antimicrobial peptides: novel insights into mechanisms of action and therapeutic prospects. Cell Mol Life Sci 74 (20), 3809-3825.

149. Maley, C.C. et al. (2017) Classifying the evolutionary and ecological features of neoplasms. Nat Rev Cancer 17 (10), 605-619.

150. Gerstung, M. et al. (2020) The evolutionary history of 2,658 cancers. Nature 578 (7793), 122-128.

151. Martincorena, I. and Campbell, P.J. (2015) Somatic mutation in cancer and normal cells. Science 349 (6255), 1483-9.

152. Raynaud, F. et al. (2018) Pan-cancer inference of intra-tumor heterogeneity reveals associations with different forms of genomic instability. PLoS Genet 14 (9), e1007669.

153. West, J. and Newton, P.K. (2019) Cellular interactions constrain tumor growth. Proc Natl Acad Sci U S A 116 (6), 1918-1923.

154. Smyth, M.J. et al. (2006) Cancer immunosurveillance and immunoediting: the roles of immunity in suppressing tumor development and shaping tumor immunogenicity. Adv Immunol 90, 1-50.

155. Fleming, A. and Allison, V.D. (1922) Observations on a Bacteriolytic Substance ("Lysozyme") Found in Secretions and Tissues. British journal of experimental pathology 3 (5), 252-260.

156. Zeya, H.I. and Spitznagel, J.K. (1963) Antibacterial and enzymic basic proteins from leukocyte lysosomes: Separation and identification. Science 142 (3595), 1085-7.

157. Ganz, T. et al. (1985) Defensins. Natural peptide antibiotics of human neutrophils. J Clin Invest 76 (4), 1427-35.

158. Lichtenstein, A. et al. (1986) In vitro tumor cell cytolysis mediated by peptide defensins of human and rabbit granulocytes. Blood 68 (6), 1407-10.

159. Larrick, J.W. et al. (1991) Complementary DNA sequence of rabbit CAP18--a unique lipopolysaccharide binding protein. Biochem Biophys Res Commun 179 (1), 170-5.

160. Curtis, K.K. et al. (2014) Novel LHRH-receptor-targeted cytolytic peptide, EP-100: first-in-human phase I study in patients with advanced LHRH-receptor-expressing solid tumors. Cancer Chemother Pharmacol 73 (5), 931-41.

161. Magana, M. et al. (2020) The value of antimicrobial peptides in the age of resistance. Lancet Infect Dis 20 (9), e216-e230.

162. Tornesello, A.L. et al. (2020) Antimicrobial Peptides as Anticancer Agents: Functional Properties and Biological Activities. Molecules 25 (12).

163. Wang, G. et al. (2016) APD3: the antimicrobial peptide database as a tool for research and education. Nucleic Acids Res 44 (D1), D1087-93.

164. Sharma, B. and Kanwar, S.S. (2018) Phosphatidylserine: A cancer cell targeting biomarker. Semin Cancer Biol 52 (Pt 1), 17-25.

165. Slaninová, J. et al. (2012) Toxicity study of antimicrobial peptides from wild bee venom and their analogs toward mammalian normal and cancer cells. Peptides 33 (1), 18-26.

166. Hoskin, D.W. and Ramamoorthy, A. (2008) Studies on anticancer activities of antimicrobial peptides. Biochim Biophys Acta 1778 (2), 357-75.

167. Chipman, D.M. and Sharon, N. (1969) Mechanism of lysozyme action. Science 165 (3892), 454-65.

168. Oram, J.D. and Reiter, B. (1968) Inhibition of bacteria by lactoferrin and other iron-chelating agents. Biochim Biophys Acta 170 (2), 351-65.

169. Huo, L. et al. (2011) Antimicrobial and DNA-binding activities of the peptide fragments of human lactoferrin and histatin 5 against Streptococcus mutans. Arch Oral Biol 56 (9), 869-76.

170. Xu, N. et al. (2008) Human alpha-defensin-1 inhibits growth of human lung adenocarcinoma xenograft in nude mice. Mol Cancer Ther 7 (6), 1588-97.

171. Kuroda, K. et al. (2015) The Human Cathelicidin Antimicrobial Peptide LL-37 and Mimics are Potential Anticancer Drugs. Front Oncol 5, 144.

172. Sveinbjørnsson, B. et al. (2017) LTX-315: a first-in-class oncolytic peptide that reprograms the tumor microenvironment. Future Med Chem 9 (12), 1339-1344.

173. Fridman, W.H. et al. (2017) The immune contexture in cancer prognosis and treatment. Nat Rev Clin Oncol 14 (12), 717-734.

174. Galon, J. and Bruni, D. (2019) Approaches to treat immune hot, altered and cold tumours with combination immunotherapies. Nat Rev Drug Discov 18 (3), 197-218.

175. Kepp, O. et al. (2020) Oncolysis without viruses - inducing systemic anticancer immune responses with local therapies. Nat Rev Clin Oncol 17 (1), 49-64.

176. Tesniere, A. et al. (2010) Immunogenic death of colon cancer cells treated with oxaliplatin. Oncogene 29 (4), 482-91.

177. Yamazaki, T. et al. (2020) PT-112 induces immunogenic cell death and synergizes with immune checkpoint blockers in mouse tumor models. Oncoimmunology 9 (1), 1721810.

178. Harrington, K. et al. (2019) Optimizing oncolytic virotherapy in cancer treatment. Nat Rev Drug Discov 18 (9), 689-706.

179. Rodriguez-Ruiz, M.E. et al. (2020) Immunological impact of cell death signaling driven by radiation on the tumor microenvironment. Nat Immunol 21 (2), 120-134.

180. McLaughlin, M. et al. (2020) Inflammatory microenvironment remodelling by tumour cells after radiotherapy. Nat Rev Cancer 20 (4), 203-217.

181. Yamazaki, T. et al. (2020) Mitochondrial DNA drives abscopal responses to radiation that are inhibited by autophagy. Nat Immunol 21 (10), 1160-1171.

182. Flood, B.A. et al. (2019) STING pathway agonism as a cancer therapeutic. Immunol Rev 290 (1), 24-38.

183. Le Naour, J. et al. (2020) Trial watch: STING agonists in cancer therapy. Oncoimmunology 9 (1), 1777624.

184. Siu, L. et al. (2020) Safety and clinical activity of intratumoral MEDI9197 alone and in combination with durvalumab and/or palliative radiation therapy in patients with advanced solid tumors. J Immunother Cancer 8 (2).

185. Marquez-Rodas, I. et al. (2020) Intratumoral nanoplexed poly I:C BO-112 in combination with systemic anti-PD-1 for patients with anti-PD-1-refractory tumors. Sci Transl Med 12 (565).

186. Pettenati, C. and Ingersoll, M.A. (2018) Mechanisms of BCG immunotherapy and its outlook for bladder cancer. Nat Rev Urol 15 (10), 615-625.

187. Shekarian, T. et al. (2019) Repurposing rotavirus vaccines for intratumoral immunotherapy can overcome resistance to immune checkpoint blockade. Sci Transl Med 11 (515).

188. Etxeberria, I. et al. (2019) Intratumor Adoptive Transfer of IL-12 mRNA Transiently Engineered Antitumor CD8(+) T Cells. Cancer Cell 36 (6), 613-629.e7.

189. Marabelle, A. et al. (2013) Intratumoral anti-CTLA-4 therapy: enhancing efficacy while avoiding toxicity. Clin Cancer Res 19 (19), 5261-3.

190. Zappasodi, R. et al. (2019) Rational design of anti-GITR-based combination immunotherapy. Nat Med 25 (5), 759-766.

191. Marabelle, A. et al. (2017) Intratumoral immunotherapy: using the tumor as the remedy. Ann Oncol 28 (suppl_12), xii33-xii43.

192. Champiat, S. et al. (2020) Intratumoral Immunotherapy: from Trial Design to Clinical Practice. Clin Cancer Res.

Highlights

- Elevated degrees of intratumoral heterogeneity are generally associated with resistance to treatment and poor disease outcome.
- Oncolytic peptides preferentially target cancer cells based on the surface properties that are rather homogeneous.
- Oncolytic peptides drive immunogenic cell death (ICD) hence promoting systemic anticancer immune responses.
- Combining oncolytic peptides with immune checkpoint inhibitors (ICIs) stands out as a promising therapeutic strategy to target ITH.

Outstanding Questions

- Would oncolytic peptides targeted to specific intracellular compartments mediate superior immunogenic effects?
- What are the molecular bases and functional consequence of the interaction between oncolytic peptides and immune cells?
- Can oncolytic peptides be engineered to include a caspase-inhibitory moiety in potential support of superior immunogenicity?
- Can specific molecules at the surface of cancer cells be targeted to limit resistance to oncolytic peptides?

Figure 1

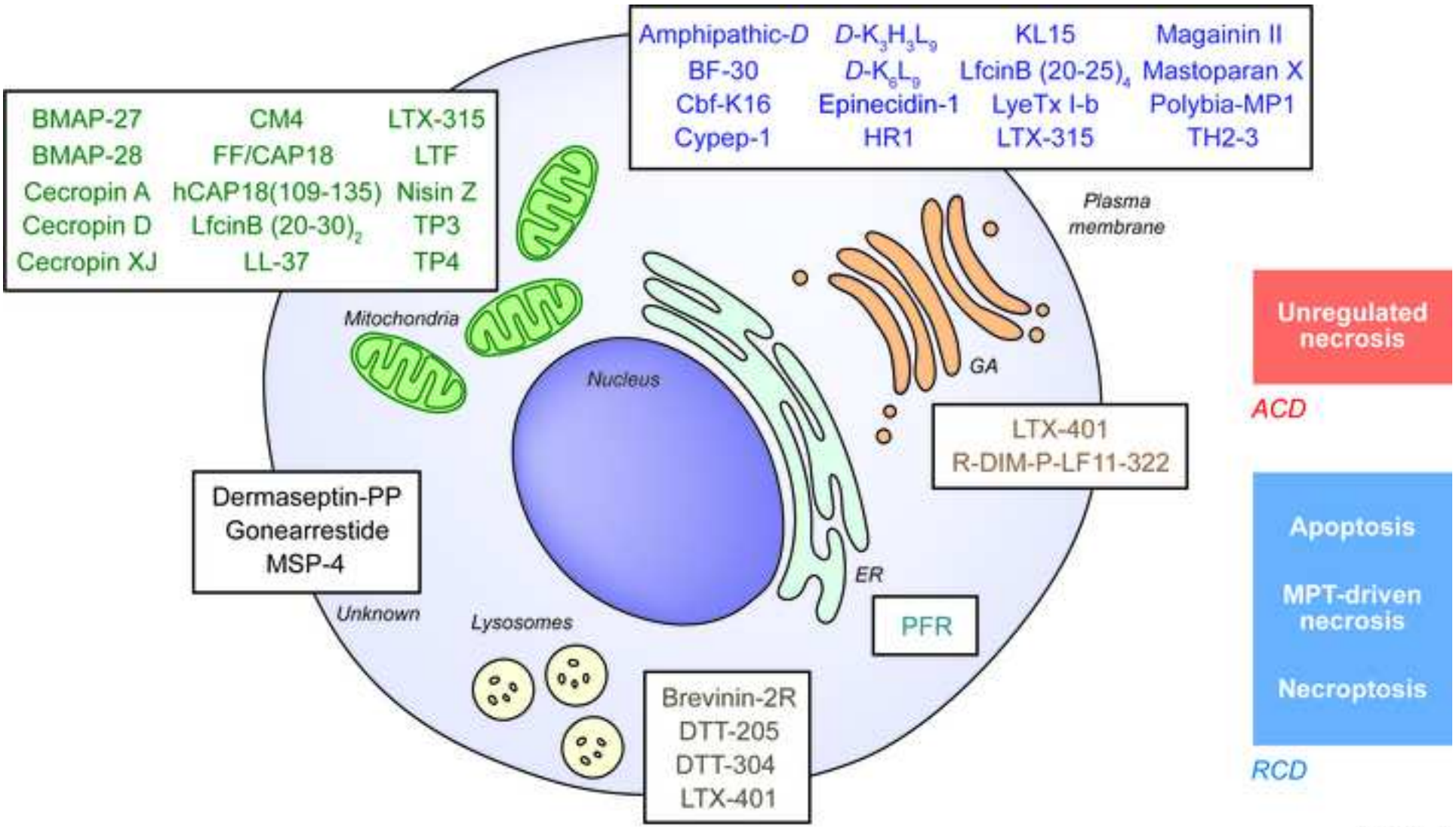

Fig. 1

Figure 2

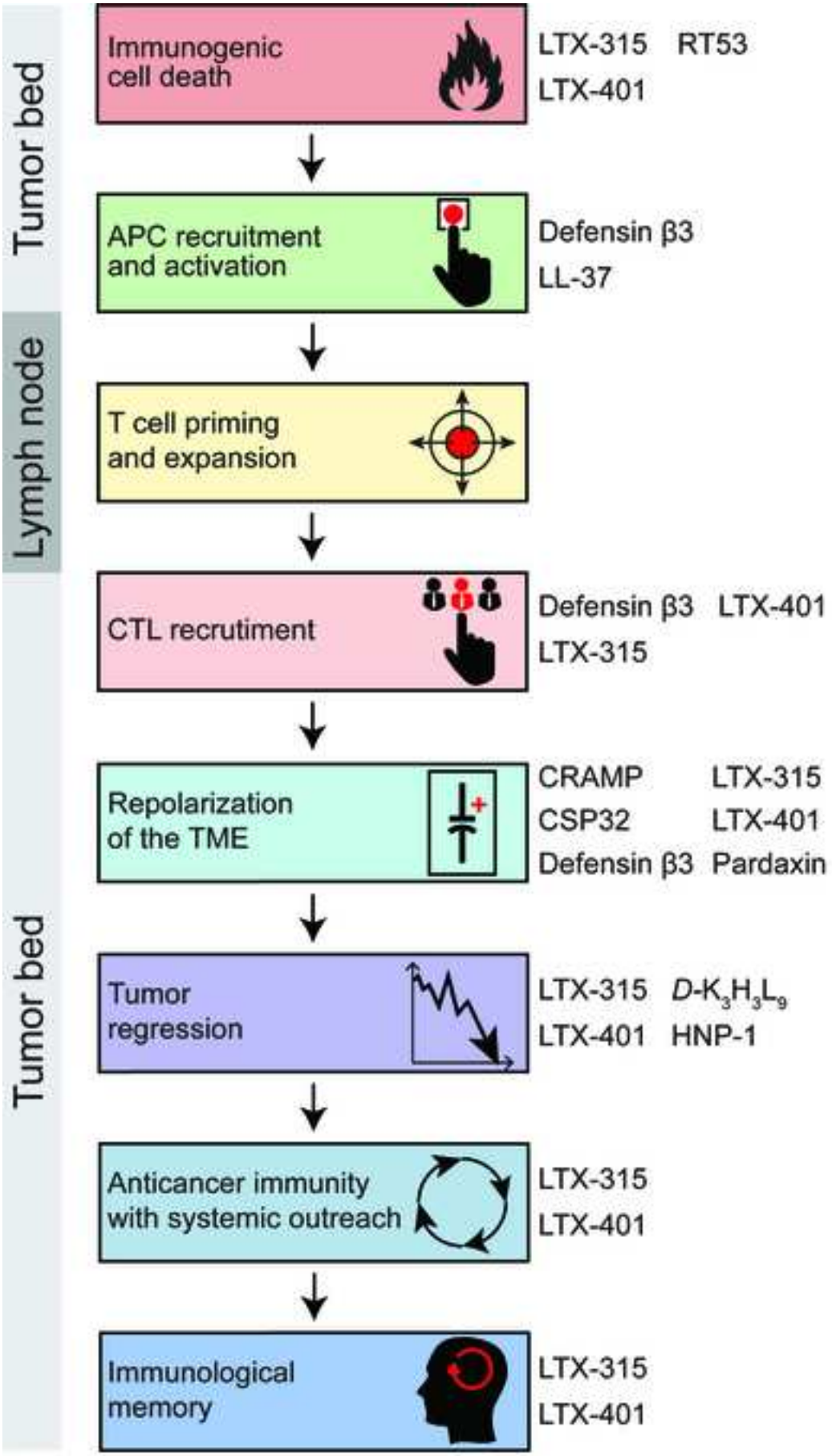

Fig. 2

Figure 3

Primary intervention	Intended effect	Antagonistic effect	Combinatorial partner
Cisplatin Doxorubicin Paclitaxel	Cytotoxicity	Drug efflux	HX-12C
Gemcitabine	Cytotoxicity	Therapeutic resistance	Mastoparan
5-fluorouracil Doxorubicin	Cytotoxicity	Cytoprotection	Nisin Z
Doxorubicin	Cytotoxicity and immunostimulation	$CD4^+$ cell exclusion	LTX-315
LL-37	Cytotoxicity and immunostimulation	Immunosuppression	TLR9 ligands
EP-100	Cytotoxicity and immunostimulation	T-cell exhaustion	ICIs
LTX-315 LTX-401	Cytotoxicity and immunostimulation	T-cell exhaustion	ICIs

Fig. 3