Opinion

T cell-mediated additive cytotoxicity – death by multiple bullets

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Immune effector cells, including cytotoxic T cells (CTLs), induce apoptosis and eliminate target cells by direct cell–cell contacts. In vivo, CTLs fail to efficiently kill solid tumor cells by individual contacts but rely upon multihit interactions by many CTLs (swarming). Recent evidence has indicated that multihit interactions by CTLs induce a series of sublethal damage events in target cells, including perforin-mediated membrane damage, induction of reactive oxygen species (ROS), nuclear envelope rupture, and DNA damage. Individual damage can be repaired, but when induced in rapid sequence, sublethal damage can accumulate and induce target cell death. Here, we summarize the sublethal damage and additive cytotoxicity concepts for CTL-induced and other cell stresses and discuss the implications for improving immunotherapy and multitargeted anticancer therapies.

Live-cell imaging reveals novel immunological processes

Over the past decade, we have witnessed unprecedented success of immunotherapies to target cancer. Multiple strategies to direct the immune system to target cancer cells have been explored, including blockade of inhibitory receptors such as CTLA-4 and PD-1/PD-L1 [1], stimulation of endogenous antitumor immunity by vaccination [2], and adoptive transfer of genetically modified T cells [3,4]. While each approach has demonstrated experimental and clinical success, no individual or combined strategy has achieved cure in the majority of patients.

Immune-checkpoint-targeted and cell-based immunotherapies aim to deliver activated cytotoxic immune cells, such as CD8 T or NK cells, to tumors and directly kill cancer cells in a cell–cell contact-dependent manner. Early observations from short time-lapse recordings of CTLs targeting B cell lymphomas suggested that individual CTLs are capable of killing target cells during individual interactions [5,6], and the killing process has since been perceived as a rapid and binary yes/no process. In addition, live-cell imaging and mathematical modeling implied that CTLs are migratory cells, and can contact and kill target cells sequentially [7–9]. However, when translated to experimental solid tumor models, monitored by intravital microscopy, effective killing of strongly antigenic tumor cells by individual CTLs remained far below the theoretically determined killing rate [10,11].

Recent single-cell microscopy recordings on CTL-mediated cytotoxicity towards virus-infected cells [12] and tumor cells in 3D culture and in vivo [13–15] have indicated that CTLs kill target cells by delivering several pulses of sublethal hits, the effect of which accumulates over time until the target cell dies. This suggests that CTLs induce damage in the target cells in an incremental manner and that target cells can repair sublethal damage and often survive the attack by cytotoxic cells. The balance between immune cell-induced damage and the damage repair capabilities of the target cell eventually determines whether the cytotoxic attack against the tumor cell is successful.

Highlights

Cytotoxic T cells (CTLs) induce a range of different damage types in tumor cells, including perforin-mediated membrane damage, nuclear envelope rupture, DNA damage and stress mediated by reactive oxygen species.

Individual damages can be sublethal, when repaired by the tumor cell.

Sublethal damage can add up until death is induced, resulting in additive cytotoxicity.

Targeted therapies that prolong CTL-mediated interactions or increase CTL density in the tumor increase the likelihood of sublethal damage accumulation and additive cytotoxicity.
Here, we discuss the types of reversible damage induced by CTLs in target cells and propose that sublethal damage and repair can be the basis of incomplete efficacy of CTL-mediated cytotoxicity. We provide examples of how frequent sublethal events can result in additive cytotoxicity and how additive effects can be perturbed by an immunocompromised environment. Finally, we discuss the implications of the concept of additive cytotoxicity for immunotherapy and molecular-targeted therapies.

Additive cytotoxicity
Recent progress in detection of molecular and structural damage in individual tumor cells and tumor cell populations has provided insight into the kinetics and consequences of CTL-mediated damage. By live-cell microscopy of fluorescent damage reporters, perforin pore formation in the membrane of antigenic tumor cells can be detected as a short-lived influx of Ca^{2+} [16,17]. In a mouse melanoma model, individual productive CTL contacts are typically survived (in 80–90% of interactions) (Figure 1A), whereas most tumor cells are eliminated after sequential interactions of multiple CTLs [13]. Statistical analysis supports the experimental observation that individual CTL contacts can induce damage of variable intensity that becomes integrated over time until either apoptosis is induced, or recovery of the target cell is achieved (Figure 1B,C). While one or two perforin events are statistically survived, three or more events increase the probability of tumor cell death induction by fourfold [13]. Similar observations of perforin pore-positive but ineffective (nonlethal) CTL–target cell pairings indicate similar multihit interactions in CD19-CAR T cells targeting mouse B cell lymphoma in vivo [18] and CTLs confronted with virally transfected target cells [12]. Furthermore, monitoring CTL killing of tumor spheroids in controlled microfluidic-based systems combined with probabilistic modeling provides evidence of cooperative killing behavior that exceeds purely additive effects of individual T cell activities [19]. Thus, CTL-induced target cell death is not a binary event but rather relies on the integration of damage frequency with damage repair efficacy in the target cell over time.

CTL-induced damage and target cell repair mechanisms
CTLs can induce various types of reversible structural and molecular damage in tumor cells (Figure 2). The induction of damage and cell death by cytotoxic immune cells may, thus, follow similar principles as observed in other types of cell stress, such as DNA double-strand breaks.
accumulating during fractionated irradiation [20,21] or nuclear damage accumulating in tumor cells navigating through confined spaces within tissues [22].

Following T cell receptor (TCR) activation by peptide–MHC complexes on the target cell surface, CTLs release the content of cytotoxic vesicles. A major component of these cytotoxic granules is granzymes, a family of serine proteases, which promote apoptosis through caspase activation and direct proteolysis of intracellular substrates, including, for example, lamin B, α-tubulin, inhibitor of caspase-activated DNase (ICAD), BH3-only protein (Bid), and DNA-dependent protein kinase (DNA-PK) [23]. To track the kinetics of granzyme-induced damage induction and repair in target cells, different fluorescent reporters have been applied, such as GCaMP6s (Ca²⁺ influx through perforin in the plasma membrane), NLS-GFP, nuclear envelope leakage; 53BP1-truncApple, DNA damage and repair; CellROX, intracellular ROS levels; H₂DCFDA, lipid peroxidation by ROS. Abbreviations: CAD, caspase-activated DNase; ER, endoplasmic reticulum; ESCRT, endosomal sorting complex required for transport; ICAD, inhibitor of caspase-activated DNase; ROS, reactive oxygen species; SPY-tubulin, fluorophore silicon rhodamine-conjugated tubulin probe. Created with BioRender.com.

**Plasma membrane damage**

Damage to the cell membrane is induced by CTL-mediated perforin pores, followed by a transient increase of intracellular Ca²⁺. While likely not being directly toxic, perforin pore-dependent Ca²⁺ elevation occurs in 50% of all CTL–melanoma cell interactions and in 90% of eventually lethal events [13]. In human melanoma cells, the Ca²⁺ influx triggers lysosome accumulation and, likely, membrane insertion at the immune synapse and resealing of the perforin-mediated membrane defects,
which increases melanoma resistance to CTL-induced killing [25,26]. Plasma membrane defects are repaired, within minutes, by internalization or shedding of the damaged site, by the endosomal sorting complex required for transport (ESCRT(III)) machinery, Rab 27 and annexin A7 [27].

Nuclear envelope rupture
CTL attack mediates damage to the nucleus by caspase-independent cleavage of proteins of the nuclear lamina, such as, for example, lamin B and nuclear matrix proteins, such as NuMa [23]. Mechanical and chemical stress can further result in rupture of the nuclear envelope [22], followed by DNA damage mediated by nuclear entry of cytoplasmic DNases. Using mouse melanoma cell lines expressing chicken ovalbumin (OVA) and transgenic TCR T cells recognizing the OVA peptide, 20% of OVA-specific CTL contacts induce nuclear envelope ruptures (visualized by leakage of NLS-GFP into the cytoplasm), the majority of which (80%) are reversible within 60 min and followed by tumor cell survival [13].

DNA damage
Cytotoxic agents, radiation, molecular-targeted therapies, and CTLs are known inducers of DNA damage followed by apoptotic cell death or, if repaired within hours to days, survival [6]. DNA double-strand breaks are sensed by DNA-binding proteins (e.g., RAD50 and 53BP1), which recruit repair complex proteins (e.g., ATM/ATR) [28,29]. Overall, 40% of antigenic CTL–tumor cell interactions cause DNA damage-associated repair foci in the nucleus, which are associated with death at ~30% probability; alternatively, DNA damage-associated foci are reversible (within hours) followed by tumor cell survival [13]. Due to its central role in securing tumor cell survival and emerging resistance, the DNA damage response (DDR) is considered an important target for improving combinations of chemo-, radio- and molecular therapy, for example, by ATM/ATR and poly(ADP-ribose) polymerase (PARP) inhibitors [30–33].

Oxidative stress
ROS are induced by CTLs in target cells, as well as by other drugs that induce cell stress [34,35]. ROS and secondary ROS products can damage any cell compartment by chemical modification and cause cell death. As an example, peroxidation of membrane lipids disrupts the plasma membrane and forms nanopores (~100 nm in diameter) [36] and causes endoplasmic reticulum (ER) stress, followed by cytoplasmic, mitochondrial, and nuclear dysfunction. Nuclear ROS can further perturb transcription and induce DNA damage. ROS are neutralized by enzymes (e.g., superoxide dismutase and catalase) and scavengers (e.g., ascorbic acid and glutathione). Because CTL-induced ROS contributes to cancer cell lethality and administration of free radical scavengers delays CTL-mediated target cell killing [37], targeted ROS modulation is being considered for enhancing cancer immunotherapies [38,39].

Mechanisms of target cell repair
The concept of additive cytotoxicity predicts that target cell susceptibility towards CTL-induced apoptosis can be countered by the activity of cell-intrinsic repair mechanisms. Using live-cell optical reporters for intracellular structural damage revealed resolution kinetics of damage signals which were in agreement with repair processes observed after mechanical and chemical challenge or ionizing radiodamage [22,40,41]. The entry of extracellular Ca2+ through perforin pores triggers rapid membrane repair mediated by membrane delivery and fusion of cytosolic vesicles at the damage site [16,25,26]. The ESCRT machinery is critical in removing perforin pores to restore membrane integrity [27]; consequently, inhibition of the ESCRT machinery increases CTL-mediated killing by two- to threefold [27]. The ESCRT machinery further contributes to restoring the nuclear lamina following mechanical rupture [41]. Nuclear envelope repair and plasma membrane repair are achieved within minutes, and this matches the repair kinetics.
observed following CTL-induced nuclear leakage. Compared with membrane repair, CTL-induced DNA double-strand breaks are more sustained, with repair times ranging from minutes to several hours [13]. These kinetics are in line with activation of DDR pathways which mediate DNA repair by nonhomologous end joining (NHEJ) and homologous recombination ranging from minutes to hours to resolve DNA damage [42].

As an emerging theme, CTLs induce intracellular damages, which accumulate over time until a detrimental, non-repairable state is reached [43]. The relative importance of each damage type and corresponding repair mechanism for inducing target cell death remains to be established.

**Implications for cytotoxic immune cell function**

The balance of damage and repair determines whether target cells survive or die, and this may have consequences for immune effector control in homeostasis and cancer disease.

**Peripheral immune checkpoint**

Additive cytotoxicity may be a mechanism that has evolved to limit tissue damage caused by autoimmunity. The multihit concept of CTL swarms predicts that a sufficient number of antigen-specific CTLs are required to induce apoptosis and that an even higher number may be required when antigen strength is low to moderate [44,45]. Thus, additive cytotoxicity induction depends on robust activation and spreading of effector CTLs and may thus represent a filter that limits undesirable cell death by rare misdirected CTLs. This may explain why autoreactive CTLs circulating in human peripheral blood occur at low frequencies without causing overt autoimmune disease [46,47].

**Immune cell cooperation**

Besides accumulation of sublethal damage by CTLs with identical TCRs, additive cytotoxicity can result from CTLs with different TCR specificities. Cooperation of polyclonal CTLs during interaction with an individual target cell may then increase the frequency of CTL encounters and thereby the chance of damage accumulation in the tumor cell. In addition, the concept of additive cytotoxicity may contribute to the specificity of polyclonal CTL response at the population level. CTLs can be directed against antigens that are also present on healthy bystander cells (e.g., gp100 and MART-1). Polyclonal CTL cooperation increases the likelihood by which tumor cells expressing multiple matching antigens receive a higher number of hits while bystander tissue cells expressing fewer CTL-specific antigens receive fewer hits and accumulate only minor damage that can be repaired. Sublethal attacks by individual cells and required additivity of damage to kill may thus reduce accidental bystander damage, a strategy which could be explored to increase safety in CAR T or TCR T cell therapies. Likewise, additive cytotoxicity may enable cooperation of cytotoxic leukocytes with complementary target-recognition strategies, such as CTLs and NK cells [48,49], CD8 and CD4 CTLs [50], or opsonin-guided macrophages [51].

**Mechanisms modulating killing efficacy and opportunities for immunotherapies**

The concept of damage accumulation opens up the possibility of modulating individual CTL–target cell contact efficacy and/or contact frequency by targeted intervention.

**Local CTL density and CTL swarming**

In 3D models and solid tumors, local tumor cell apoptosis rates are positively correlated with local CTL density [52] and CTL migration activity [53]. Within the concept of additive cytotoxicity, both CTL density and motility increase sequential sublethal encounters with tumor cells. In microfluidic models that allow analysis of interactions between tumor spheroids and CTLs with high spatio-temporal control, CTL clustering enhances their ability to kill by a positive feedback mechanism.
that enhances local CTL swarming [19], possibly by chemokines produced by CTLs upon target cell recognition [54]. In addition, supramolecular attack particles, which were recently described to be deposited on tumor cells by transient CTL and NK cell contacts [55–57], may contribute to the additive cytotoxic effects towards target cells. Increasing CTL infiltration and swarming can further be achieved by normalizing dysfunctional tumor vessels to facilitate transendothelial migration [58,59] or by enhancing local CTL proliferation and/or retention by stabilizing contacts to tumor cells through activating immune checkpoints [14]. As an aggregated effect, additive cytotoxicity could underlie the reduced tumor growth in response to combined anti-CTLA-4 and radiotherapy, which enhances both local CTL density and contact duration in breast carcinoma [60].

Contact stability and tuning of single-hit interactions
The minimum lethal dose, that is, the damage type and strength required to induce apoptosis, depends on both the magnitude of damage caused by each cytotoxic vesicle degranulation event and the frequency of sequential hits. Live-cell recordings in vitro and in vivo noted a positive correlation of lethal interactions with longer contact duration [14], increased number of sublethal events within one contact [13], and only short time gaps (below 50 min) between sequential damage events (Figure 1C). The number of cytotoxic granules released by CTLs per contact may vary, but typically one hit does not deplete the granule pool and serial hits by the same CTL in subsequent encounters or towards the same target cell are possible [61,62]. Consequently, stabilizing contacts may enhance the number of exocytosis events and improve the apoptosis rate of single CTL–target cell interactions. Physiologically, mechanical properties of the microenvironment such as stiffness of tumor cells and matrix may promote stable immune synapse formation and killing efficacy [63,64]. Immune synapse stability may also be tunable by molecular targeting, for example, by bispecific antibodies that connect CTLs and target cells and facilitate repetitive hits [65,66]. In conclusion, stabilizing the cytolytic synapse may increase the frequency of hits delivered by the same or more stably engaging CTLs.

Metabolic perturbation
In a metabolically perturbed tumor microenvironment, CTL effector function can be compromised by deregulated redox homeostasis, deficient mitochondrial respiration, as well as dysfunctional \( \text{Ca}^{2+} \) release-activated \( \text{Ca}^{2+} \) (CRAC) channels [15]. Perturbation of CRAC channel function, which is a frequent consequence of disturbed vessel function and hypoxia in solid tumors, dampens the calcium-dependent activation of CTLs and leads to delayed CTL degranulation and a reduction of sublethal hit frequency delivered to the target cell [15]. Thus, normalizing the metabolically disturbed tumor microenvironment will increase CTL efficacy on the single-cell level.

Inhibiting target cell repair activity
To shift the balance between CTL-induced damage and the repair capacity of the target cell towards apoptosis, tumor cell resistance can be decreased by targeted interference with the repair processes. For example, inhibition of the ESCRT membrane repair complex prolongs the membrane damage caused by individual CTLs by two- to threefold and considerably increases CTL-mediated killing of tumor cells [27]. Furthermore, similar to chemo- and radio-sensitizing approaches, tumor cell susceptibility to sublethal CTL damage may be increased by combining complementary treatment modalities to induce DNA stress. For example, adoptive CTL transfer can be combined with DDR inhibitors which target members of the DNA-PK or the PARP family [67].

Concluding remarks and future perspectives
Despite high molecular specificity of cellular immune responses, killing of solid tumor cells is an inefficient process with a high failure rate at the single-cell level. Contrary to textbook knowledge,
recent studies have highlighted that CTL-mediated killing is not a binary yes/no process but instead relies on an incremental sequence of sublethal damage events delivered by one or multiple CTLs. When recorded by sensitive damage reporters using single-cell microscopy, sublethal events represent structural cell damage in tumor cells that is often repaired before apoptosis is completed. Thus, a sublethal hit can be classified as the minimum damage unit caused by CTLs. Different types of damage may concur and their efficacy relies on the cell-specific activity of their respective repair mechanisms, with analogies to apoptosis resistance observed in sublethal radio- or chemotherapy-induced cell stress. Incomplete or faulty repair of sublethal damage may carry the risk to increase cancer aggressiveness [68,69], highlighting the therapeutic importance of achieving lethal immunotargeting. As resistance to CTL attack occurs downstream to antigen recognition and lytic granule secretion, the efficacy of even highly specific engineered cytototoxic immune cell or activated immune cells following immune checkpoint modulation may be compromised by enhanced repair ability of tumor cells. Better characterization of involved molecular pathways and identification of potential interference strategies to prevent repair or to increase additive cytotoxicity are therefore an important future challenge for cancer immunotherapy and combination therapies (see Outstanding questions).

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No interests are declared.

References
1. Sharma, P. and Allison, J.P. (2015) The future of immune checkpoint therapy. Science 348, 56–61
2. Haen, S.P. et al. (2022) Towards new horizons: characterization, classification and implications of the tumour antigenic repertoire. Nat. Rev. Clin. Oncol. 17, 596–610
3. Chandran, S.S. and Kebanoff, C.A. (2019) T cell receptor-based cancer immunotherapy: emerging efficacy and pathways of resistance. Immuno. Rev. 290, 127–147
4. Schmidts, A. and Maus, M.V. (2018) Making CAR T cells a solid option for solid tumors. Front. Immunol. 9, 2593
5. Stritchcombe, J.C. et al. (2001) The immunological synapse of CTL contains a secretory domain and membrane bridges. Immunity 15, 751–761
6. Perelstein, A.S. and Belt, G.J. (1982) Delivery of lethal hits by cytotoxic T lymphocytes in multilacellular conjugates occurs sequentially but at random times. J. Immunol. 129, 2796–2801
7. Cheu, P.J. and Mitchison, T.J. (2013) Imaging burst kinetics and spatial coordination during serial killing by single natural killer cells. Proc. Natl. Acad. Sci. U. S. A. 110, 6488–6493
8. Isaaz, S. et al. (1995) Serial killing by cytotoxic T lymphocytes: T cell receptor triggers degranulation, re-filling of the lytic granules and secretion of lytic proteins via a non-granule pathway. Eur. J. Immunol. 25, 1071–1079
9. Foley, M.H. et al. (2014) High avidity CD8+ T cells efficiently eliminate mobile HIV-infected targets and execute a locally focused program of anti-viral function. PLoS One 9, e87923
10. Engelhardt, J.J. et al. (2012) Marginating dendritic cells of the tumor microenvironment cross-present tumor antigens and stably engage tumor-specific T cells. Cancer Cell 21, 402–417
11. Breit, B. et al. (2009) Two-photon imaging of intratumoral CD8+ T cell cytotoxic activity during adoptive T cell therapy in mice. J. Clin. Invest. 118, 1390–1397
12. Halle, S. et al. (2016) In vivo killing capacity of cytotoxic T cells is limited and involves dynamic interactions and T cell cooperativity. Immunity 44, 233–243
13. Weigelin, B. et al. (2021) Cytotoxic T cells are able to efficiently eliminate cancer cells by additive cytotoxicity. Nat. Commun. 12, 5217
14. Weigelin, B. et al. (2015) Focusing and sustaining the antitumor CTL effector killer response by agonist anti-CD137 mAb. Proc. Natl. Acad. Sci. U. S. A. 112, 7551–7556
15. Staats, J. et al. (2021) Metabolic screening of cytotoxic T-cell effector function reveals the role of crac channels in regulating lethal hit delivery. Cancer Immunol. Res. 9, 526–538
16. Keefe, D. et al. (2005) Perforin triggers a plasma membrane-repair response that facilitates CTL induction of apoptosis. Immunity 23, 249–262
17. Lopez, J. et al. (2013) Perforin forms transient pores on the target cell plasma membrane to facilitate rapid access of granzymes during killer cell attack. Blood 121, 2659–2668
18. Khazen, R. et al. (2021) Functional heterogeneity of cytotoxic T cells and tumor resistance to cytotoxic hits limit anti-tumor activity in vivo. EMBO J. 40, e106658
19. Ponteix, G. et al. (2022) High resolution microfluidic assay and probabilistic modeling reveal cooperation between T cells in tumor killing. Nat. Commun. 13, 25–28
20. Schmid, Z. et al. (2019) DNA damage accumulation during fractionated low-dose radiation compromises hippocampal neurogenesis. Radiat. Oncol. 137, 45–54
21. Rocker, L. et al. (2014) Even low doses of radiation lead to DNA damage accumulation in lung tissue according to the genetically-defined DNA repair capacity. Radiat. Oncol. 111, 212–218
22. Denis, C.M. et al. (2016) Nuclear envelope rupture and repair during cancer cell migration. Science 352, 353–358
23. Cullen, S.P. and Martin, S.J. (2008) Mechanisms of granule-dependent killing. Cell Death Differ. 15, 251–262.
24. Halle, S. et al. (2017) Mechanisms and dynamics of T cell-mediated cytotoxicity in vivo. Trends Immunol. 38, 432–443.
25. Fisk, L. et al. (2022) Ultra-rapid lytic granule release from CTLs activates CD8+ dependent synaptic resistance pathways in melanoma cells. Sci. Adv. 8, eabk3234.
26. Khazen, R. et al. (2016) Melanoma cell lysosome secretory burst neutralizes the CTL-mediated cytotoxicity at the lytic synapse. Nat. Commun. 7, 10923.
27. Ritter, A.T. et al. (2022) ESCRT-mediated membrane repair protects tumor-derived cells against T cell attack. Science 376, 377–382.
28. Blackford, A.N. and Jackson, S.P. (2017) ATM, ATR, and DNA-PK: the trinity at the heart of the DNA damage response. Mol. Cell 66, 801–817.
29. Panier, S. and Boulton, S.J. (2014) Double-strand break repair: DNA-dependent protein kinase comes into focus. Nat. Rev. Microbiol. 12, 1–8.
30. O’Connor, M.J. (2015) Targeting the DNA damage response in cancer. Mol. Cell 60, 547–560.
31. Weber, A.M. and Ryan, A.J. (2015) ATM and ATR as therapeutic targets in cancer. Pharmacol. Ther. 149, 1–18.
32. Durant, S.T. et al. (2018) The brain-penetrant clinical ATM inhibitor AZD1390 radiosensitizes and improves survival of preclinical brain tumor models. Sci. Adv. 4, eaat1719.
33. Zhou, Q. et al. (2021) Inhibition of ATM induces hypersensitivity to proton irradiation by upregulating toxic end joining. Cancer Res. 81, 3333–3346.
34. Pardo, J. et al. (2004) Apoptotic pathways are selectively activated by granulocyte A and/or granulocyte B in CTL-mediated target cell lysis. J. Cell Biol. 167, 457–468.
35. Martinvalet, D. et al. (2005) Granulyme A induces caspase-independent mitochondrial damage, a required first step for apoptosis. Immunity 22, 355–370.
36. Ferrari, C.S. et al. (2020) Fusion of lysosomes to plasma membrane initiates radiation-induced apoptosis. J. Cell Biol. 219, e201903176.
37. Jacquemin, G. et al. (2015) Granulyme B-induced mitochondrial ROS are required for cell death. Cell Death Differ. 22, 862–874.
38. Sisir, B.J. et al. (2021) Avasopassen synergizes with hypofractionated radiation to ablate tumors through the generation of hydrogen peroxide. Sci. Transl. Med. 13, eabj7756.
39. Majumder, D. et al. (2017) Catalse inhibition an anti-cancer property of flavonoids: a kinetic and structural evaluation. Int. J. Biol. Macromol. 104, 929–935.
40. Cullen, E. et al. (2019) SRBF1 mediates productive and mutagenic DNA repair through distinct phosphoribosyl interactions. Cell 153, 1266–1280.
41. Raab, M. et al. (2016) ESCRT II repairs nuclear envelope rup-
tures during cell migration to limit DNA damage and cell death. Science 352, 359–362.
42. Scully, R. et al. (2019) DNA double-strand break repair-pathway choice in somatic melanoma cells. Nat. Rev. Mol. Cell Biol. 20, 698–712.
43. McKenzie, B. et al. (2022) Greek fire, poison arrows, and scorpion bombs: how tumor cells defend against the siege weapons of cytotoxic T lymphocytes. Front. Immunol. 13, 894506.
44. Moreau, H.D. et al. (2015) Signal strength regulates antigen-mediated T cell deceleration by distinct mechanisms to promote local exploration or arrest. Proc. Natl. Acad. Sci. U. S. A. 112, 12151–12156.
45. Sykulev, Y. et al. (1996) The law of mass action governs antigen-stimulated cytolytic activity of CD8+ cytotoxic T lymphocytes. Proc. Natl. Acad. Sci. U. S. A. 92, 11990–11992.
46. Bournaud, C. et al. (2000) Impact of negative selection on the T cell repertoire reactive to a self-peptide: a large fraction of T cell clones escapes clonal deletion. Immunol. 13, 829–840.
47. Yu, W. et al. (2015) Clonal deletion prunes but does not eliminate self-specific αβ TCR T lymphocytes. Immunol. 42, 929–941.
48. Kühnapp, F.J. et al. (2015) NK cells and CD8+ T cells cooperate to improve therapeutic responses in melanoma treated with interleukin-2 (IL-2) and CTLA-4 blockade. J. Immunother. Cancer 3, 18.
49. Friedman, K.S. et al. (2020) Interdependence of CTL and NK cell cytotoxicity against melanoma cells. bioRxiv. Published online November 14, 2020. https://doi.org/10.1101/2020.11.14.56072.
50. Cachot, A. et al. (2021) Tumor-specific cytolytic CD4 T cells mediate immunity against human cancer. Sci. Adv. 7, eabe3348.
51. Alvey, C.M. et al. (2017) SRPA-inhibited, marrow-derived mac-
rrophages engorge, accumulate, and differentiate in antibody-targeted regression of solid tumors. Curr. Biol. 27, 2005–2077.
52. Busch, S. et al. (2013) CD8 T cell concentration determines their efficiency in killing cognate antigen-expressing syngeneic mammalian cells in vitro and in mouse tissues. J. Exp. Med. 207, 223–235.
53. Salmon, H. et al. (2012) Matrix architecture defines the preferen-
tial localization and migration of T cells into the stroma of human lung tumors. J. Clin. Invest. 122, 889–910.
54. Niño, J.L.G. et al. (2020) Cytotoxic T cells swim by homotypic chemokinetic signaling. eLife 9, e56554.
55. Böltín, A. et al. (2020) Supramolecular attack particles are autonomous killing entities released from cytotoxic T cells. Science 368, 897–901.
56. Ambrose, A.R. et al. (2020) Synaptic secretion from human natural killer cells is diverse and includes supramolecular attack particles. Proc. Natl. Acad. Sci. U. S. A. 117, 23717–23720.
57. Chang, H.F. et al. (2022) Identification of distinct cytotoxic granules as the origin of supramolecular attack particles in T lymphocytes. Nat. Commun. 13, 1059.
58. Shintani, R.K. et al. (2011) Antiangiogenic agents can increase lymphocyte infiltration into tumor and enhance the effectiveness of adoptive immunotherapy of cancer. Cancer Res. 70, 6171–6180.
59. Johansson, A. et al. (2012) Tumor-targeted TNFα stabilizes tumor vessels and enhances active immunotherapy. Proc. Natl. Acad. Sci. U. S. A. 109, 7841–7846.
60. Rucco, M.G. et al. (2012) Suppressing T cell motility induced by anti-CTLA-4 monoclonal improves antitumor effects. J. Clin. Invest. 122, 2718–2730.
61. Weidemann, A. et al. (2008) Cytotoxic T lymphocytes kill multiple targets simultaneously via a chemoattractant uncoupling of lytic and stim-
ulatory synapses. Proc. Natl. Acad. Sci. U. S. A. 105, 10965–10970.
62. Canamero, I. et al. (2009) Visualizing CTL melanoma cell interactions: multiple hits must be delivered for tumour cell annihilation. J. Cell. Mol. Med. 13, 3834–3846.
63. Hirvonen, C. and Saltielis, M. (2016) Biophysical aspects of T lympho-
cyte activation at the immune synapse. Front. Immunol. 7, 46.
64. Baiz, R. et al. (2016) Cytotoxic T cells sense and attack cells using a single chain antibody construct. J. Immunol. 195, 98–104.
65. Frankel, S.R. and Basuvar, P.A. (2013) Targeting T cells to tumor cells using bispecific antibodies. Curr. Opin. Chem. Biol. 17, 385–392.
66. Huang, R.X. and Zhou, P.K. (2020) DNA damage response signaling pathways and targets for radiotarget sensitization in cancer. Sig. Transduct. Target. Ther. 5, 60.
67. Berenheit, K. et al. (2020) Failed apoptosis enhances melanoma cancer cell aggressiveness. Cell Rep. 31, 107731.
68. Hawkins, C.J. and Miles, M.A. (2021) Mutagenic consequences of sublethal cell death signaling. Int. J. Mol. Sci. 22, 6144.