Biochemical mechanisms implemented by human acute myeloid leukaemia cells to suppress host immune surveillance

Inna M. Yasinska[1], Isabel Gonçalves Silva[1], Svetlana Sakhnevych[1], Bernhard F. Gibbs[2], Ulrike Raap[2], Elizaveta Fasler-Kan[3,4], Vadim V. Sumbayev[1]*

1 – Medway School of Pharmacy, Universities of Kent and Greenwich, Chatham Maritime, United Kingdom

2 - Department of Dermatology and Allergy, University of Oldenburg, Germany

3 - Department of Pediatric Surgery and Department of Clinical Research, Children's Hospital, Inselspital, University of Bern, Switzerland

4 – Department of Biomedicine University Hospital Basel and University of Basel, Switzerland

* **- To whom correspondence should be addressed:** Vadim V. Sumbayev: V.Sumbayev@kent.ac.uk

Human malignant tumours have developed a variety of effective molecular strategies which allow them to escape host immune surveillance leading to a disease progression. This includes haematological malignancies such as acute myeloid leukaemia (AML), a blood/bone marrow cancer originating from self-renewing myeloid cell precursors which rapidly becomes systemic. AML cells are capable of escaping immune attack despite being permanently exposed to host immune cells including cytotoxic T cells (CTCs) and natural killer (NK) cells[1]. AML cells successfully implement biochemical mechanisms which allow them to inactivate cytotoxic lymphoid cells (NK cells and CTCs) upon direct contact as well as at a distance[2]. In this case they not only "fight back" against immune cells but also effectively prevent the actual process of cytotoxic immune attack. In this work we will discuss several important biochemical mechanisms which allow AML cells to form immunological synapses with cytotoxic lymphoid cells and also comprehensively inactivate anti-cancer immunity at a distance.

T helper (Th) type cells generate and secrete interleukin-2 (IL-2), a stimulatory cytokine which triggers activation of NK cells as well as CTCs[3]. Upon activation, these cytotoxic lymphoid cells become capable of attacking malignant (AML for example) cells delivering the proteolytic enzyme granzyme B into them. Granzyme B itself can directly activate one of the key apoptotic enzymes, caspase-3. However, granzyme B performs cleavage of the pro-apoptotic protein Bid, forming its active form tBid which negatively impacts on mitochondrial function, inducing release of cytochrome c, one of the major components of electron respiratory chain. Cytochrome c interacts with apoptotic protease activating factor-1 (Apaf-1) and pro-caspase-9 thus forming an apoptosome, which induces programmed death of target cell[2].

It has become evident that AML cells are capable of expressing surface proteins such as programmed death-1 (PD-1) receptor ligands (PD-Ls) 1 and 2 as well as CD86, the ligand of

cytotoxic T-cell antigen 4 (CTLA4)[1]. T helpers and CTCs/NK cells express PD-1 receptors on their surface. AML cell surface-based PD-1 ligands 1 and 2 (PD-L(i)) interact with PD-1 on lymphoid cell surfaces. As a result, T helper cells stop producing IL-2 required for the activation of both CTCs and NK cells. PD-1 signalling attenuates the protein kinase C θ (PKC-θ) loop thus preventing activation of transcription factors NF-κB and AP-1, which are required for IL-2 production[4].

On the other hand, the interaction of PD-Ls with PD-1 on the surface of NK cells and CTCs leads to their rapid inactivation, and, as a result, they lose ability to kill AML cells[1]. In addition, AML cells are often capable of expressing the CTLA4 ligand CD86. Interaction of CD86 with CTLA4 rapidly leads to inactivation of effector T cells[1,5].

Thus, one could conclude that CD86 and PD-Ls 1 and 2 are involved in the formation of immunological synapses with both regulatory and cytotoxic lymphoid cells leading to downregulation of the biochemical activation of CTCs and NK cells. Direct interaction of PD-Ls and CD86 with CTCs and NK cells leads to loss of their anti-cancer activities. Schematically this process is shown in Figure 1.

Recent evidence also demonstrated the ability of AML cells to downregulate the activity of cytotoxic lymphoid cells through lymphocyte-activation gene 3 (LAG-3), which is a homolog of CD84. AML cells were reported to induce exhaustion of cytotoxic lymphoid cells through LAG-3 but detailed mechanisms of this event remain to be elucidated[1,5].

Recently, it has become evident that the immune receptor Tim-3 (T cell immunoglobulin and mucin domain containing protein 3) is involved in protecting AML cells against host immune surveillance[2,6]. Tim-3 has a natural ligand galectin-9 (a tandem protein which contains two receptor-binding domains fused together by a peptide linker) which was suggested to form an autocrine loop with the receptor[7]. When present on the cell surface, galectin-9 induces Tim-3

downstream signalling which includes activation of pathways responsible for cell survival[7-9]. This first of all includes activation of transcription factor nuclear factor kappa B (NF-kB)[7], translational pathways controlled by mammalian target of rapamycin (mTOR) and hypoxic signalling required for the adaptation of AML cells to stress conditions and their survival in general[8,9]. The Tim-3-galectin-9 complex was also reported to activate the β-catenin pathway which, together with NF-kB, controls self-renewal of AML cells[7]. Taken together, one may conclude that galectin-9 mediates survival signalling through Tim-3 (Figure 2).

Galectin-9 lacks a secretory domain and thus requires a trafficker in order to be taken to the cell surface and then secreted[2,10]. We have recently found that AML cells but not healthy leukocytes express the neuronal receptor latrophilin 1 (LPHN1). LPHN1 is expressed in haematopoietic stem cells, but disappears upon maturation unless they undergo malignant transformation into AML cells. Using its natural ligands (for example fibronectin leucine rich transmembrane protein 3, FLRT3), LPHN1 facilitates exocytosis of Tim-3-galectin-9, which then triggers cell survival signalling. However, Tim-3 either on its own or in complex with galectin-9 can also be proteolytically shed from the surface of AML cells thus leading to secretion of both proteins. Galectin-9 interacts with NK cells and CTCs (most likely though Tim-3)[2]. This leads to impairing of cytotoxic activity of NK cells and killing of CTCs. Interestingly, NK cells produce interferon gamma (IFN-γ) in response to stimulation with galectin-9. IFN-γ induces the activation of indoleamine 2,3-dioxygenase (IDO1), an enzyme converting L-tryptophan into formyl-L-kynurenine, which is then degraded into L-kynurenine and released[12]. L-kynurenine impairs the cytotoxic activity of NK cells[2].

Intriguingly, IFN-γ is also known to induce the expression of PD-Ls [13], which might further promote the ability of AML cells to protect themselves against host immune surveillance.

Soluble Tim-3 released by AML cells is capable of downregulating IL-2 secretion by Th cells acting *via* a receptor which remains to be identified[2]. This prevents the activation of cytotoxic lymphoid cells. Importantly, the secretion of Tim-3 and galectin-9 allows AML cells to supress cytotoxic lymphoid cells at a distance thus minimising direct interaction with them. This allows AML cells to "focus on" self-renewal thus leading to rapid disease progression. The functioning of the Tim-3-galectin-9 secretory and signalling pathway in AML cells is summarised in Figure 2.

Importantly, stress associated with the events described above leads to release of high mobility group box 1 (HMGB1) protein by AML cells which finally triggers production of interleukin 1 beta (IL-1β) by healthy leukocytes[14]. IL-1β was reported to induce the expression and production of stem cell factor (SCF) by epithelial cells via mTOR pathway and hypoxic signalling[15]. SCF is a major hematopoietic growth factor that controls the AML progression thus becoming highly oncogenic[15]. In such a way, AML cells employ body systems to produce factors required for their proliferation/disease progression[14,15].

Taken together, it is clear that AML cells implement comprehensive mechanisms in order to escape immune surveillance and progress the disease. Pharmacological targeting of the biochemical pathways responsible for immune escape will enable the human immune system to potentially cure AML and thus avoid aggressive chemotherapy and bone marrow transplantation. Therefore, design and development of new strategies for anti-AML immunotherapy are a major focus for current applied AML research. It is also vital to investigate whether other cancers operate similar mechanisms since certain solid tumours (e.g. colon cancer[16]) were already reported to use the Tim-3-galectin-9 loop for immune evasion.

References

1. Sehgal A, Whiteside TL, Boyiadzis M. Programmed death-1 checkpoint blockade in acute myeloid leukemia. *Expert Opin Biol Ther* 2015, **15:** 1191-1203.
2. Goncalves Silva I, Yasinska IM, Sakhnevych SS, Fiedler W, Wellbrock J, Bardelli M, *et al.* The Tim-3-galectin-9 Secretory Pathway is Involved in the Immune Escape of Human Acute Myeloid Leukemia Cells. *EBioMedicine* 2017, **22:** 44-57.
3. Geng H, Zhang GM, Li D, Zhang H, Yuan Y, Zhu HG, *et al.* Soluble form of T cell Ig mucin 3 is an inhibitory molecule in T cell-mediated immune response. *J Immunol* 2006, **176:** 1411-1420.
4. Karwacz K, Bricogne C, MacDonald D, Arce F, Bennett CL, Collins M, *et al.* PD-L1 co-stimulation contributes to ligand-induced T cell receptor down-modulation on CD8+ T cells. *EMBO Mol Med* 2011, **3:** 581-592.
5. Kursunel MA, Esendagli G. A Co-Inhibitory Alliance in Myeloid Leukemia: TIM-3/Galectin-9 Complex as a New Target for Checkpoint Blockade Therapy. *EBioMedicine* 2017, **23:** 6-7.
6. Jin HT, Anderson AC, Tan WG, West EE, Ha SJ, Araki K, *et al.* Cooperation of Tim-3 and PD-1 in CD8 T-cell exhaustion during chronic viral infection. *Proc Natl Acad Sci USA* 2010, **107:** 14733-14738.
7. Kikushige Y, Miyamoto T, Yuda J, Jabbarzadeh-Tabrizi S, Shima T, Takayanagi S, *et al.* A TIM-3/Gal-9 Autocrine Stimulatory Loop Drives Self-Renewal of Human

Myeloid Leukemia Stem Cells and Leukemic Progression. *Cell Stem Cell* 2015, **17:** 341-352.

8. Prokhorov A, Gibbs BF, Bardelli M, Ruegg L, Fasler-Kan E, Varani L, *et al.* The immune receptor Tim-3 mediates activation of PI3 kinase/mTOR and HIF-1 pathways in human myeloid leukaemia cells. *Int J Biochem Cell Biol* 2015, **59:** 11-20.
9. Goncalves Silva I, Gibbs BF, Bardelli M, Varani L, Sumbayev VV. Differential expression and biochemical activity of the immune receptor Tim-3 in healthy and malignant human myeloid cells. *Oncotarget* 2015, **6:** 33823-33833.
10. Goncalves Silva I, Ruegg L, Gibbs BF, Bardelli M, Fruehwirth A, Varani L, *et al.* The immune receptor Tim-3 acts as a trafficker in a Tim-3/galectin-9 autocrine loop in human myeloid leukemia cells. *OncoImmunology* 2016, **5:** e1195535.
11. Sumbayev VV, Goncalves Silva I, Blackburn J, Gibbs BF, Yasinska IM, Garrett MD, *et al.* Expression of functional neuronal receptor latrophilin 1 in human acute myeloid leukaemia cells. *Oncotarget* 2016, **7:** 45575-45583.
12. Folgiero V, Cifaldi L, Li Pira G, Goffredo BM, Vinti L, Locatelli F. TIM-3/Gal-9 interaction induces IFNgamma-dependent IDO1 expression in acute myeloid leukemia blast cells. *J Hematol Oncol* 2015, **8:** 36.
13. Norde WJ, Maas F, Hobo W, Korman A, Quigley M, Kester MG, *et al.* PD-1/PD-L1 interactions contribute to functional T-cell impairment in patients who relapse with cancer after allogeneic stem cell transplantation. *Cancer Res* 2011, **71:** 5111-5122.
14. Yasinska IM, Gonzalves Silva I, Sakhnevych SS, Ruegg L, Hussain R, Siligardi G, *et al.* High mobility group box 1 (HMGB1) acts as an “alarmin” to promote acute myeloid leukaemia progression. *OncoImmunology* 2018, **7**: e1438109
15. Wyszynski RW, Gibbs BF, Varani L, Iannotta D, Sumbayev VV. Interleukin-1 beta induces the expression and production of stem cell factor by epithelial cells: crucial

involvement of the PI-3K/mTOR pathway and HIF-1 transcription complex. *Cell Mol Immunol* 2016, **13**: 47-56.

16. Kang CW, Dutta A, Chang LY, Mahalingam J, Lin YC, Chiang JM, *et al.* Apoptosis of tumor infiltrating effector TIM-3+CD8+ T cells in colon cancer. *Scientific reports* 2015, **5:** 15659.

Figure Legends

Figure 1. AML cells supress IL-2 production and the activity of cytotoxic lymphoid cells *via* PD-1 and CTLA4 receptors

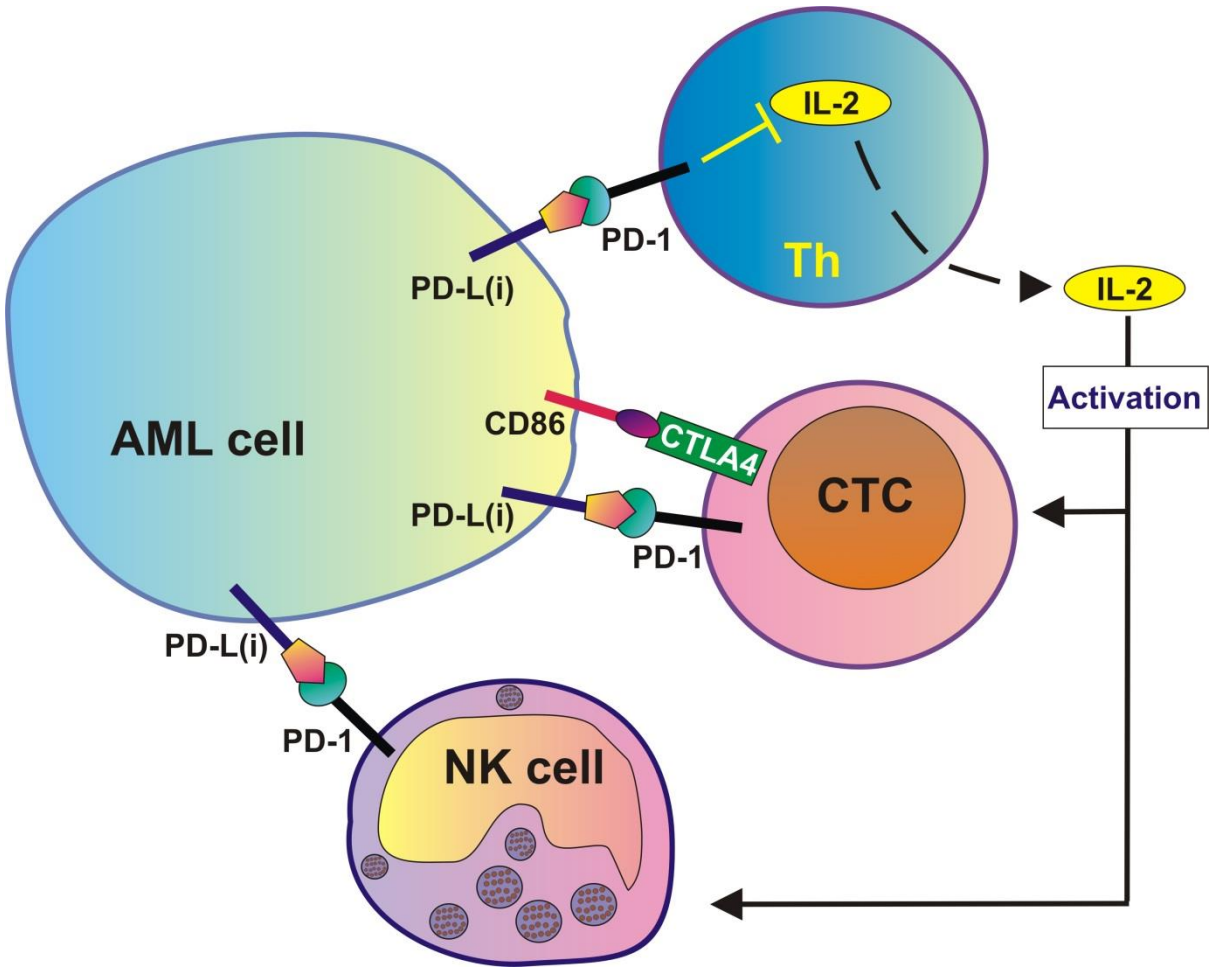

Figure 2. The Tim-3-galectin-9 pathway regulates both intracellular AML cell survival signalling and immune escape.