antibodies against IL-22 have been developed for this disease. The suggestion that avoiding overactivation of signaling pathways through cytokine receptors with a light touch to treat inflammatory diseases without losing beneficial effects that result from low-level tonic cytokine signaling is exciting and will require further study.

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CONFLICT OF INTEREST
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REFERENCES

Dumoutier L, de Meester C, Tavernier J, Renauld JC. New activation modus of STAT3: a tyrosine-less region of the interleukin-22 receptor recruits STAT3 by interacting with its coiled-coil domain. J Biol Chem 2009;284:26377–84.

Fauny M, Moulin D, D'Amico F, Netter P, Petitpain N, Arnone D, et al. Paradoxical gastrointestinal effects of interleukin-17 blockers. Ann Rheum Dis 2020;79:1132–8.

Fujino S, Andoh A, Bamba S, Ogawa A, Hata K, Araki Y, et al. Increased expression of interleukin 17 in inflammatory bowel disease. Gut 2003;52:65–70.

Li Z, Brown MA. Progress of genome-wide association studies of ankylosing spondylitis. Clin Transl Immunology 2017;6:e163.

Liu J, Chang HW, Huang ZM, Nakamura M, Sekhon S, Ahn R, et al. Single-cell RNA sequencing of psoriatic skin identifies pathogenic Tc17 cell subsets and reveals distinctions between CD8$^+$ T cells in autoimmunity and cancer. J Allergy Clin Immunol 2021;147:2370–80.

Ma HL, Liang S, Li J, Napierata L, Brown T, Benoit S, et al. IL-22 is required for Th17 cell-mediated pathology in a mouse model of psoriasis-like skin inflammation. J Clin Invest 2008;118:597–607.

Michiels C, Puigdevall L, Cochez P, Achouri Y, Cheou P, Hendrickx E, et al. A targetable, noncanonical signal transducer and activator of transcription 3 activation induced by the Y-Less region of IL-22 receptor orchestrates imiquimod-induced psoriasis-like dermatitis in mice. J Invest Dermatol 2021;141:2668–78.e6.

Van Belle AB, de Heusch M, Lemaire MM, Hendrickx E, Warnier G, Dunussi-Joannopoulos K, et al. IL-22 is required for imiquimod-induced psoriasiform skin inflammation in mice. J Immunol 2012;188:462–9.

Whibley N, Gaffen SL. Gut-busters: IL-17 ain't afraid of no IL-23. Immunity 2015;43:620–2.

Wolk K, Kunz S, Witte E, Friedrich M, Asadullah K, Sabat R. IL-22 increases the innate immunity of tissues. Immunity 2004;21:241–54.

See related article on pg 2690

A Possible Role for PAI-1 Blockade in Melanoma Immunotherapy

Mario Del Rosso[1], Gabriella Fibbi[1], Anna Laurenzana[1], Francesca Margheri[1] and Anastasia Chillà[1]

In their new article in the *Journal of Investigative Dermatology*, Tseng et al. (2021) confirm that the sensitivity of melanoma cells to anti–PD-L1 checkpoint inhibitor therapy is correlated with high PD-L1 surface expression. By blocking PD-L1 membrane clearing, controlled by LRP1 and PAI-1, the expression of high-cell-surface levels of PD-L1 was maintained.

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Background and novelty

Immune checkpoint inhibitors (ICIs) have changed the landscape of cancer treatment. In particular, the PD-1 and PD-L1 immune checkpoint molecules are significantly involved in the evasion of tumors from immune control, and the use of anti–PD-L1 mAbs has revolutionized the treatment of various types of cancer, including melanoma. However, despite initial enthusiasm, several clinical trials have shown that although treatment with anti–PD-L1 mAbs in advanced melanoma showed higher, more durable responses in a subset of patients, there was no difference between survival and that observed when conventional chemotherapy was used (Wang et al., 2017). In their new article in the *Journal of Investigative Dermatology*, Tseng et al. (2021) show that targeting extracellular PAI-1 through the PAI-1 inhibitor tiplaxtinin (TPX) synergizes with anti–PD-L1 checkpoint blockade in a model of murine melanoma, thus paving the way for potentially more effective melanoma treatment. This work stems from two different lines of previous investigations indicating that improved clinical outcomes in patients with melanoma subjected to anti–PD-L1 therapy are associated with high PD-L1 positivity (Patel and Kurzrock, 2015) and that a temporary inhibition of PD-L1 and EGFR–clathrin–mediated endocytosis potentiated the response of human tumors to ICIs (Chew et al., 2020). As reported in this article, using immunohistochemical analysis of a significant number of paraffin-embedded sections from patients with melanoma, Tseng et al. (2021) showed that individuals with low PAI-1 expression and high levels of PD-L1 were associated with early melanoma stages (stages I and II), whereas high PAI-1/PD-L1 ratios were associated with late stages (stages III and IV), supporting the data obtained in vitro and in animals.

Following up on the observation that extracellular PAI-1 promoted the internalization of surface-expressed PD-L1 in melanoma cells by inducing clathrin-mediated endocytosis, Tseng et al. (2021) tested the hypothesis that PD-L1 was translocated to lysosomes by PAI-1–dependent mechanisms, showing that such transport is mediated by LRP1.

Role of LRP1 in the regulation of extracellular proteolysis and PD-L1

LRP1 is a very sociable multipurpose molecule that belongs to the family of

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Clinical Implications

- High-grade melanomas exhibit lower surface expression of PD-L1.
- There is an inverse relationship between PAI-1 levels and anti–PD-L1 efficacy in patients with melanoma.
- PAI-1 blockade could enhance the efficacy of anti–PD-L1 therapy in patients with melanoma.

receptors for low-density lipoproteins. LRP1 (also known as CD91) interacts with >35 ligands, and it has been identified as the receptor for α2M-proteinase complex and heat shock proteins on antigen-presenting cells (Etique et al., 2013). LRP1 (Figure 1) is characterized by an extracellular α-chain, which interacts with extracellular matrix proteins, proteases, protease-inhibitor complexes, GFs, and proteins involved in lipid metabolism. The transmembrane β-chain stimulates the endocytosis of molecules linked to the α-chain and activates signaling systems (Etique et al., 2013). From a general standpoint, LRP1 keeps in check the extent of extracellular proteolysis. In this context, the control of PAI-1 activity plays an important role in the regulation of extracellular fibrinolysis linked to the activity of uPA and tPA. The mechanism is simple (Figure 1): after the interaction of uPA with its cognate receptor uPAR (which is in turn associated with membrane integrins), PAI-1 binds to uPA and neutralizes its proteolytic activity. The complex interacts with both α- and β-chains of LRP1. Notably, each member of the cell-associated fibrinolytic system may interact with LRP1 in the absence of other components with lower affinity. The incorporation of PAI-1 leads to one-order-of-magnitude higher interactions, suggesting that the binding of proteinases to PAI-1 uncovers cryptic binding sites with a high affinity for LRP1 (Kounnas et al., 1993).

Although LRP1 is present in clathrin-coated pits as well as in caveolae (Boucher et al., 2002), Tseng et al. (2021) show that the internalization of the LRP1 multimolecular complexes occurs by inducing clathrin-coated pits–dependent endocytosis when PAI-1 is involved, suggesting a tight coregulation of cell surface PD-L1 that has previously been reported to undergo clathrin- but not caveolin-mediated endocytosis (Li et al., 2018). The receptors forming part of the internalization complex (LRP1 and uPAR) are recycled to the cell membrane, whereas the ligands (uPA, tPA, PAI-1) are degraded in lysosomes (Etique

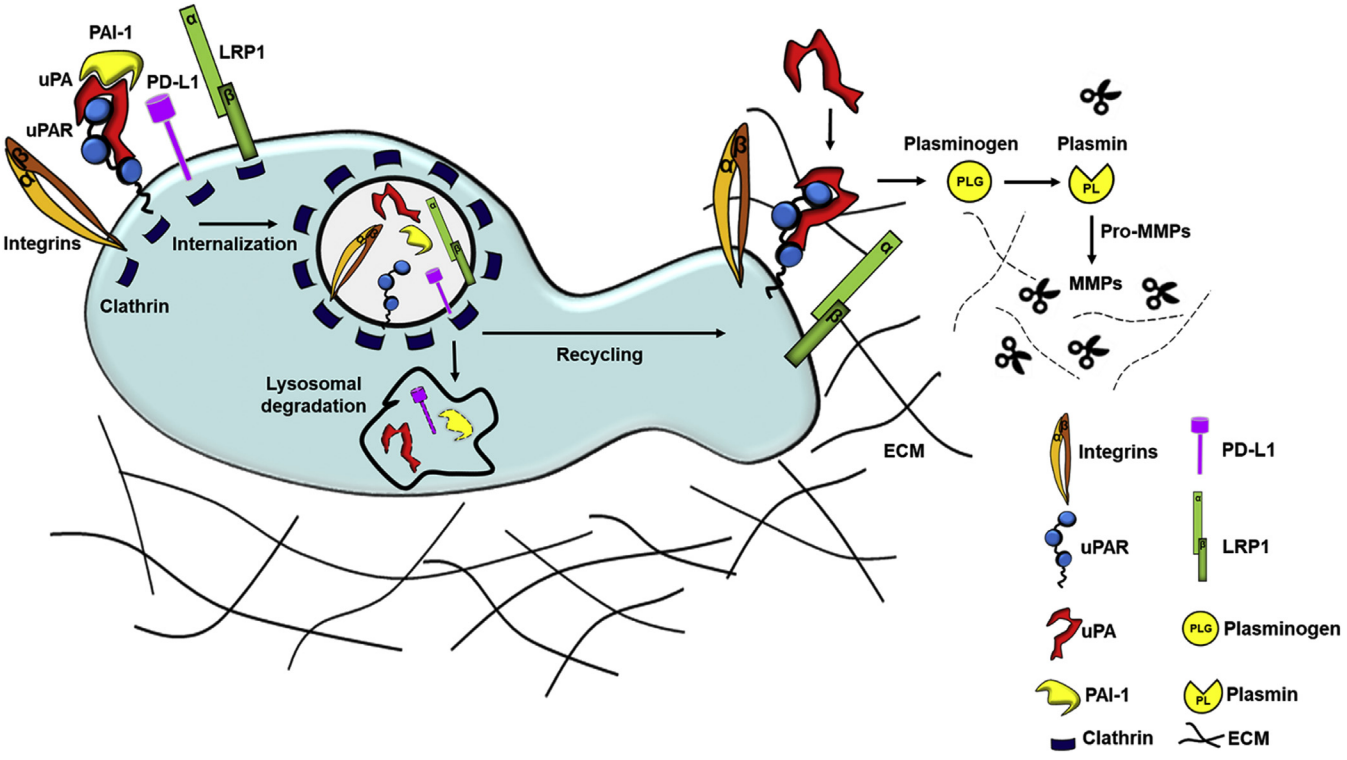

Figure 1. Molecular dynamics associated with low-density LRP-1 Both PAI-1 alone and the complex formed by uPAR/uPA/PAI-1 interact with the α-chain of LRP1 in membrane domains where clathrin is present in the inner side of the membrane and PD-L1 on the outer surface. Activation of the β and α chains of LRP1 leads to an internalization of the entire molecular complex and the fusion of clathrin-covered vesicles with lysosomes. uPA, PAI-1, and PD-L1 are degraded in lysosomes (thereby leading to a decrease in PD-L1), whereas uPAR, LRP1, and integrins are recycled to the cell surface. In particular, the interaction of uPAR with uPA activates a new cycle of degradation of the ECM, mediated both by uPA-dependent activation of plasminogen to plasmin and by the plasmin-dependent activation of the MMPs, thus favoring the invasiveness and metastasis of neoplastic cells. ECM, extracellular matrix; MMP, matrix metalloprotease.

et al., 2013), undergoing the same fate as PD-L1 (Figure 1).

Tseng et al. (2021) have concluded that coincident with LRP1-mediated endocytosis of PAI-1, PD-L1, which is also associated with clathrin-coated pits, is internalized in endosomal vesicles and is directed to lysosomes for degradation, leading to the decreased expression on the surfaces of melanoma cells and consequent desensitization to therapy with specific PD-L1 inhibitors.

Inhibition of PAI-1

Tseng et al. (2021) used various approaches to block endocytosis of the LRP1 receptor complex (and PD-L1)—ranging from inhibiting transport from the endoplasmic reticulum to the Golgi complex to blocking microtubule polymerization or function of the actin filaments—and ultimately chose to study TPX, a low-molecular-weight inhibitor of PAI-1. Although TPX was not promising in clinical trials owing to difficulties in inherent bleeding disorders (Brown, 2010), Tseng et al. (2021) successfully used it both in vitro and in vivo in a syngeneic model of melanoma, showing that TPX inhibition of PAI-1–dependent endocytosis blocks lysosomal degradation of PDL-1, maintains a high surface expression of PDL-1, and enhances the tumor-suppressive activity of anti–PD-L1 blockade. These results are very encouraging and may pave the way for intensified research on PAI-1 inhibitors with a more favorable benefit-to-risk ratio.

Wider perspectives

As underlined by Tseng et al. (2021), the pleiotropic properties of PAI-1 extend beyond the inverse relationship with the expression of PD-L1, involving the wider field of the basis of malignancy, particularly the promotion of angiogenesis and the control of tumor invasion and metastasis. Virtually all types of human cancers, including melanoma, are characterized by more or less relevant alterations of the molecules of the membrane-associated fibrinolytic system. Bearing in mind that PAI-1 has been validated as a negative prognostic factor for clinical use in level-of-evidence-1 studies in mammary carcinoma (Duffy et al., 2014) and that it has been shown to negatively impact the prognosis of patients with melanoma (Tseng et al., 2021), the discovery of the regulation of PDL-1 expression by inhibition of PAI-1 phagocytosis lays the foundations for the development of new drugs that may have wide-ranging effects on the natural history of human cancers.

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CONFLICT OF INTEREST

The authors state no conflict of interest.

REFERENCES

Boucher P, Liu P, Gotthardt M, Hiesberger T, Anderson RG, Herz J. Platelet-derived growth factor mediates tyrosine phosphorylation of the cytoplasmic domain of the low density lipoprotein receptor-related protein in caveolae. J Biol Chem 2002;277:15507–13.

Brown NJ. Therapeutic potential of plasminogen activator inhibitor-1 inhibitors. Ther Adv Cardiovasc Dis 2010;4:315–24.

Chew HY, De Lima PO, Gonzalez Cruz JL, Banushi B, Echejoh G, Hu L, et al. Endocytosis inhibition in humans to improve responses to ADCC-mediating antibodies. Cell 2020;180:895–914.e27.

Duffy MJ, McGowan PM, Harbeck N, Thomssen C, Schmitt M. uPA and PAI-1 as biomarkers in breast cancer: validated for clinical use in level-of-evidence-1 studies. Breast Cancer Res 2014;16:428.

Etique N, Verzeaux L, Dedieu S, Emonard H. LRP-1: a checkpoint for the extracellular matrix proteolysis. Biomed Res Int 2013;2013:152163.

Kounnas MZ, Henkin J, Argraves WS, Strickland DK. Low density lipoprotein-related protein/alpha 2-macroglobulin receptor mediates cellular uptake of pro-urokinase. J Biol Chem 1993;268:21862–7.

Li CW, Lim SO, Chung EM, Kim YS, Park AH, Yao J, et al. Eradication of triple-negative breast cancer cells by targeting glycosylated PD-L1. Cancer Cell 2018;33:187–201.e10.

Patel SP, Kurzrock R. PD-L1 expression as a predictive biomarker in cancer immunotherapy. Mol Cancer Ther 2015;14:847–56.

Tseng Y-J, Lee C-H, Chen W-Y, Yang J-L, Tzeng H-T. Inhibition of PAI-1 blocks PD-L1 endocytosis and improves the response of melanoma cells to immune checkpoint blockade. J Invest Dermatol 2021;141:2690–98.e6.

Wang DY, Eroglu Z, Ozgun A, Leger PD, Zhao S, Ye F, et al. Clinical features of acquired resistance to anti-PD-1 therapy in advanced melanoma. Cancer Immunol Res 2017;5:357–62.