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## Acquired resistance in pancreatic cancer: characterization and exploration of actionable targets of a multifactorial disease

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# Chapter 5

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## Targeting Integrins for Cancer Therapy – Disappointments and Opportunities

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### Abstract

Integrins mediate adhesive interactions between cells and their environment, including neighboring cells and extracellular matrix (ECM). These heterodimeric transmembrane receptors bind extracellular ligands with their globular head domains and connect to the cytoskeleton through multi-protein interactions at their cytoplasmic tails. Integrin containing cell–matrix adhesions are dynamic force-responsive protein complexes that allow bidirectional mechanical coupling of cells with their environment. This allows cells to sense and modulate tissue mechanics and regulates intracellular signaling impacting on cell faith, survival, proliferation, and differentiation programs. Dysregulation of these functions has been extensively reported in cancer and associated with tumor growth, invasion, angiogenesis, metastasis, and therapy resistance. This central role in multiple hallmarks of cancer and their localization on the cell surface make integrins attractive targets for cancer therapy. However, despite a wealth of highly encouraging preclinical data, targeting integrin adhesion complexes in clinical trials has thus far failed to meet expectations. Contributing factors to therapeutic failure are 1) variable integrin expression, 2) redundancy in integrin function, 3) distinct roles of integrins at various disease stages, and 4) sequestering of therapeutics by integrin-containing tumor-derived extracellular vesicles. Despite disappointing clinical results, new promising approaches are being investigated that highlight the potential of integrins as targets or prognostic biomarkers. Improvement of therapeutic delivery at the tumor site via integrin binding ligands is emerging as another successful approach that may enhance both efficacy and safety of conventional therapeutics. In this review we provide an overview of recent encouraging preclinical findings, we discuss the apparent disagreement between preclinical and clinical results, and we consider new opportunities to exploit the potential of integrin adhesion complexes as targets for cancer therapy.

### Introduction

#### *Integrin Structure*

Integrins represent a family of transmembrane adhesion receptors, facilitating the adhesive connection between cells and their surrounding extracellular matrix (ECM) or neighboring cells<sup>1–3</sup>. They comprise a group of heterodimeric proteins generated by non-covalent association of an  $\alpha$ - and a  $\beta$ -subunit<sup>4</sup>. Both subunits are classified as type 1 transmembrane proteins, composed of a rather large extracellular domain and a relatively small transmembrane and intracellular region<sup>4,5</sup>. The globular head domain creates a binding site for extracellular ligands while the short cytoplasmic tails interact with a cluster of associated proteins that ultimately connects to the cytoskeleton. In total there are 18  $\alpha$ - and eight  $\beta$ -subunits, generating 24 different heterodimers, known to be expressed in humans<sup>5</sup>. This variety in combinations allows integrins to interact with—and respond to a broad range of ligands, including insoluble ECM proteins, matricellular proteins, cell surface proteins, and soluble proteins<sup>6</sup>. Several recognition motifs for integrin-binding have been identified. The Arg-Gly-Asp (RGD) motif is recognized by eight different integrins and has been found in a plethora of molecules ranging from ECM proteins to growth factors to coats of microorganisms.

#### *Integrin Function*

Integrin transmembrane receptors execute two core functions: they mediate adhesion of cells to the ECM or neighboring cells, and they engage in transduction of signals received from the microenvironment. Integrin-mediated cell adhesion is dynamic: flexibility in integrin conformation allows a balance between active (open; high affinity) and inactive (closed; low affinity) states. The active state is regulated by interaction of the intracellular adaptor proteins talin and kindlin with the  $\beta$ -subunit cytoplasmic tail and is further stabilized by interaction with ligand at the extracellular integrin head domain<sup>5,7</sup>. Moreover, firm cell adhesion requires integrins to cluster in cell adhesion complexes that connect to the cytoskeleton.

Integrin-mediated cell adhesion controls many aspects of cell behavior including survival, proliferation, metabolism, differentiation, as well as cell shape and motility<sup>8</sup>. Several mechanisms of such outside-in signaling have been proposed. First, integrins allow cells to interact with the ECM in which soluble growth factors such as VEGF, TGF $\beta$  and many others are concentrated, modified, and presented to cells<sup>9</sup>.

Second, integrins can directly bind and activate growth factors such that they can stimulate their cognate receptors, a process currently established for activation of TGF $\beta$  by  $\alpha$ v $\beta$ 6 and  $\alpha$ v $\beta$ 8<sup>10</sup>. Third, integrin engagement and clustering can lead to local activation of receptors for soluble ligands such as EGF, PDGF, and others, often involving receptor crosstalk via Src family kinases<sup>11,12</sup>. Fourth, the dynamic intracellular complex of adaptor and signaling proteins that couples integrins to the cytoskeleton allows 1) local signaling through GTPases and kinases and 2) sensing of and responding to mechanical aspects of the microenvironment by mechanoresponsive interactions<sup>8,13</sup>.

### *Integrins in Cancer*

Dysregulation of integrin expression on cancer cells has been extensively studied in cell culture and animal models and shown to provide therapeutic opportunities for arresting tumor growth reducing resistance to chemo- or radiotherapy, or attenuating invasion and metastasis. Studies using genetically engineered mouse models or using human tumor cells transplanted in immune deficient mice have extensively shown that deletion of integrins in cancer cells or preventing integrin function with blocking antibodies or peptides could interfere with tumor growth, metastasis, and resistance to chemo- or radiotherapy<sup>14–18</sup>. For the large family of  $\beta$ 1 integrins, dual roles have been identified in growth versus metastasis, indicating that caution is warranted for their application as therapeutic targets<sup>19–22</sup>. Integrins such as  $\alpha$ v $\beta$ 3,  $\alpha$ v $\beta$ 5, and  $\alpha$ 5 $\beta$ 1, are not only expressed on tumor cells but are also induced on endothelial cells during the process of angiogenesis<sup>23,24</sup>. These integrins have indeed been shown to serve as targets for anti-angiogenic therapies in cancer, although the mode of action of anti-angiogenic drugs targeting integrins remains enigmatic<sup>23–26</sup>.

Recent studies have added a range of novel emerging cancer-related processes that require the participation of integrins, including the establishment of a pre-metastatic niche, epithelial-to-mesenchymal transition (EMT), metabolic rewiring, cancer cell stemness and dormancy<sup>27–34</sup>. The involvement of integrin  $\alpha$ v $\beta$ 6 in activation of TGF $\beta$  was recently connected to SOX4 mediated cancer immune evasion:  $\alpha$ v $\beta$ 6 blocking antibodies could inhibit SOX4 expression and sensitize mouse models for triple negative breast cancer to T cell mediated killing in response to immune checkpoint inhibitors<sup>35</sup>. Integrin  $\alpha$ v $\beta$ 8, which can also activate TGF $\beta$ , represents a target expressed on immune cells for modulating anti-tumor immunity. I.e.,  $\alpha$ v $\beta$ 8 blocking antibodies or specific depletion of integrin  $\alpha$ v $\beta$ 8 from the surface of CD4<sup>+</sup>CD25<sup>+</sup>

regulatory T cells could attenuate TGF $\beta$  mediated inhibition of CD8<sup>+</sup> T cells and thereby restore tumor killing capacity of CD8<sup>+</sup> T cells and synergize with radio- or immune therapy <sup>36</sup>.

The expression of integrins on the cell-surface and their apparent role in several cancer related processes makes them appealing targets for the development of cancer therapies. However, despite the abundance of promising preclinical data, integrin targeting therapies in clinical studies have thus far largely failed to deliver. Notably, although not within the scope of this review, components of the integrin signaling complexes represent additional targets in cancer. For example, focal adhesion kinase (FAK) is overexpressed or activated in multiple cancers and supports tumor cell proliferation, migration, and therapy resistance. Small molecule inhibitors targeting FAK, such as defactinib, GSK2256098, VS-6063, and BI 853520, are currently being investigated in several clinical trials, mostly in combination with other agents <sup>37,38</sup>. Src is another interesting target associated with integrin signaling. Dasatinib, a Src inhibitor, showed efficacy when combined with docetaxel in castration-resistant prostate cancer patients<sup>39</sup> (NCT00439270), and was more effective than imatinib in Pediatric Philadelphia Chromosome–Positive Acute Lymphoblastic Leukemia <sup>40</sup>. On the other hand, dasatinib monotherapy failed to meet expectations in patients with recurrent glioblastoma <sup>41</sup> or in patients with locally advanced or stage IV mucosal, acral, or vulvovaginal melanoma <sup>42</sup>. The challenges of targeting Src family proteins were recently reviewed by Martellucci and others <sup>43</sup>. Integrins interact with many other cytoplasmic proteins, which are being investigated for their potential as therapeutic targets, however these have not yet been translated to the clinic <sup>44,45</sup>.

In this review we focus on integrins as drug targets in cancer and discuss the apparent disagreement between preclinical and clinical results, we provide an overview of new encouraging preclinical findings and consider new opportunities to exploit the potential of integrin adhesion complexes in the effective treatment of cancer.

### **Finalized clinical trials exploring integrin therapeutics**

Monoclonal antibodies and synthetic RGD peptides have been used in clinical trials to target integrins <sup>46</sup>. These drugs typically block integrin function by occupying the ligand binding site. Integrin blocking antibodies previously showed efficacy in different diseases, such as multiple sclerosis, thrombosis prevention after percutaneous coronary intervention, ulcerative colitis and Chron's disease <sup>47</sup>. Moreover, in multiple preclinical studies, inhibition of  $\alpha\beta3$ ,  $\alpha\beta5$  or  $\beta1$  integrins prevented tumor

angiogenesis, reduced tumor growth and limited metastatic spread, supporting the translation of these antibodies and blocking peptides into the clinic for cancer therapy<sup>48–51</sup>. Despite promising preclinical results, such therapeutics did not make it to the market. Therapeutic safety was often not the bottleneck for integrin targeting therapeutics. The major drawback was their lack of efficacy (Table 1).

The majority of integrin directed therapeutics in clinical trials involve antibodies or peptides targeting  $\alpha v$ -integrins and these have thus far failed to show benefit for cancer patients. The integrin  $\alpha v$  antibody abituzumab was used in a phase II trial to treat patients with metastatic castration-resistant prostate cancer<sup>52</sup> (NCT01360840). Even though a reduction in prostate cancer associated-bone lesion development was observed in the antibody treated group of patients, the primary endpoint of progression free survival (PFS) was not significantly extended. Interestingly, the addition of abituzumab to the standard of care did show some beneficial effect on the overall survival of a subset of metastatic colorectal carcinoma patients<sup>53,54</sup> (NCT01008475).

## Targeting Integrins for Cancer Therapy

Clinical trial identifier	Phase	Name therapeutic	Type therapeutic	Target integrin	Combination therapy with	Condition	Result	Mode of action
NCT01360840	II	Abituzumab (EMD525797)	Antibody	$\alpha V$	—	Metastatic Castration-Resistant Prostate cancer	PFS not significantly different	Blocks cell adhesion
NCT01008475	I/II	Abituzumab (EMD525797)	Antibody	$\alpha V$	Cetuximab Irinotecan	Metastatic colorectal cancer	PFS not significantly different	Blocks cell adhesion
NCT00246012	II	Inte-tumumab (CNTO 95)	Antibody	$\alpha V$	Dacarbazine	Stage IV Melanoma	PFS not significantly different	Blocks ligand binding site
NCT00537381	II	Inte-tumumab (CNTO 95)	Antibody	$\alpha V$	Docetaxel Prednisone	Metastatic Hormone Refractory Prostate Cancer	All efficacy endpoints better in placebo	Blocks ligand binding site
	II	Vitaxin (MEDI-523)	Antibody	$\alpha V\beta 3$	—	Metastatic cancers	No tumor regression	Blocks ligand binding site
	II	Etaracizumab (MEDI-522, Abegrin)	Antibody	$\alpha V\beta 3$	Dacarbazine	Stage IV metastatic melanoma	PFS not significantly different	Blocks ligand binding site
NCT00842712 NCT00121238 NCT00705016	II	Cilengitide (EMD 121974)	Inhibitory peptide	$\alpha V\beta 3/\alpha V\beta 5$	Multiple combinations	Multiple cancers	No benefits compared to standard of care	Blocks ligand binding site
NCT00689221	III	Cilengitide (EMD121974)	Inhibitory peptide	$\alpha V\beta 3/\alpha V\beta 5$	Temozolomide + Radiotherapy	Newly Diagnosed Glioblastoma	Median OS not significantly different	Blocks ligand binding site
NCT00401570 NCT00654758 NCT00516841 NCT00635193 NCT00369395 NCT00100685	I/II	Volociximab (M200)	Antibody	$\alpha V\beta 1$	Alone or in combination with standard of care	Metastatic Pancreatic Cancer, Non-Small Cell Lung Cancer, Ovarian and Peritoneal cancer, Renal cell carcinoma	Partial or no significant effects	Blocks ligand binding site



NCT00675428	II	Natalizumab	Antibody	VLA-4, ( $\alpha 4$ )	—	Multiple myeloma	Terminated due to low enrollment	Allosteric inhibition
NCT00131651 NCT00352313	I/II	ATN-161	Small peptide antagonist	$\alpha 5\beta 1$	Alone or in combinations	Glioma, renal cancer and other solid tumors	No therapeutic benefits	Blocks ligand binding site; prevents interaction with fibronectin synergy site
NCT01313598	I	GLPG0187	Non-peptide Integrin antagonist	Arg-Gly-Asp (RGD)-binding integrins	—	Solid tumors	No effect	Blocks ligand binding site

**Table 1.** Overview of failed or terminated major clinical trials for the assessment of integrin targeting therapeutics in cancer.

Another phase II  $\alpha v$  targeting study illustrated that a combination treatment of dacarbazine with the monoclonal  $\alpha v$ -antibody intetumumab did not enhance treatment efficacy over monotreatment in patients with stage IV melanoma<sup>55</sup> (NCT00246012). Testing this antibody in a phase II trial with prostate cancer patients did not improve outcome either<sup>56</sup> (NCT00537381). Antibodies specific for  $\alpha v\beta 3$  integrin have been extensively evaluated in clinical trials as well<sup>46</sup>. In a phase I trial, the  $\alpha v\beta 3$ -antibody vitaxin failed to show benefit for patients with metastatic solid tumors<sup>57</sup>. The additional effect of the  $\alpha v\beta 3$ -antibody etaracizumab was assessed on top of dacarbazine treatment in stage IV melanoma patients<sup>58</sup>, however no significant differences in the time to progression (TTP) or PFS were observed. Several phase II trials have explored efficacy of the  $\alpha v\beta 3/\alpha v\beta 5$ -selective function blocking peptide cilengitide for treatment of solid tumors alone or in combination with other therapies, but results were not encouraging<sup>6,59–61</sup> (NCT00842712, NCT00121238, NCT00705016). Likewise, cilengitide failed to improve therapeutic efficacy in combination with standard of care for patients with newly diagnosed glioblastoma in a phase III trial<sup>62</sup> (NCT00689221).

Other integrins that have been targeted include  $\alpha 5 \beta 1$ . Unfortunately, phase I and II trials using the small peptide antagonist of integrin  $\alpha 5 \beta 1$  ATN-161 have thus far not shown benefit for glioma patients or in other solid tumors <sup>63</sup>(NCT00131651, NCT00352313). Similarly, the combination treatment of gemcitabine with the  $\alpha 5 \beta 1$  chimeric monoclonal antibody volociximab did not show any additional treatment efficacy over gemcitabine monotreatment in metastatic pancreatic cancer patients in a phase II trial <sup>64</sup> (NCT00401570). Moreover, volociximab efficacy was not encouraging in peritoneal, ovarian, non-small cell lung cancer or melanoma <sup>65–69</sup> (NCT00401570, NCT00654758, NCT00516841, NCT00635193, NCT00369395, NCT00100685). Natalizumab, an antibody targeting  $\alpha 4 \beta 1$  (VLA-4) has shown promising clinical results in autoimmune related diseases such as multiple sclerosis and Crohn's disease <sup>70,71</sup>. However, a phase 1/2, two-arm dose-finding study of natalizumab for relapsed or refractory Multiple Myeloma, was unfortunately terminated due to insufficient patient enrolment (NCT00675428). Among the therapeutics discussed so far, natalizumab is the only one not targeting the ligand binding site. Instead, it acts through allosteric interactions <sup>72</sup>. Further exploring such alternative forms of integrin receptor pharmacology may lead to new and more effective treatments <sup>73</sup>.

### Ongoing clinical trials exploring integrin therapeutics

As discussed, clinical trials of  $\alpha v$ -integrin inhibitors or drugs targeting other integrins have thus far not been encouraging. Other approaches are now being explored in new clinical trials (Table 2).

A phase I trial aims to treat patients with previously treated pancreatic cancer or other solid tumors with the anti- $\alpha v \beta 3$  protein, ProAgio (NCT05085548). ProAgio binds  $\alpha v \beta 3$  outside the classical ligand-binding site. Instead of blocking ligand binding, it triggers recruitment and activation of caspase 8, resembling a mechanism previously associated with unligated integrins <sup>74,75</sup>. This may lead to apoptosis in tumor cells, endothelial cells, and cancer-associated fibroblasts (CAF) with increased expression of  $\alpha v \beta 3$ . Subsequently, this can result in a reduction of the stroma density of pancreatic cancer patients increasing access of conventional anti-cancer therapeutics to the tumor.

In a planned phase I trial, the safety, tolerability and efficacy of the integrin  $\beta 6$  targeting antibody-drug conjugate SGN-B6A will be studied in patients with advanced solid tumors. SGN-B6A consists of an antibody targeting integrin  $\beta 6$  conjugated with

monomethyl auristatin E, an antimitotic agent that induces apoptosis by binding to tubulin <sup>76</sup>(NCT04389632). A randomized phase II trial, planned at the end of 2021 will study efficacy of a tumor penetrating iRGD peptide, CEND-1, in combination with gemcitabine and nab-paclitaxel in patients with metastatic pancreatic cancer. The first-in-class agent CEND-1 binds tumor cells and enhances delivery of co-administered anti-cancer agents. In a recently completed phase I clinical trial the safety and efficacy of CEND-1 was already explored <sup>77,78</sup>. Based on the trial data, the combination treatment was regarded as safe. Importantly, efficacy of this treatment exceeded the efficacy of the mono-treatments, with ongoing progression free survival of some patients.

## Targeting Integrins for Cancer Therapy

Clinical trial identifier	Phase	Name therapeutic	Type therapeutic	Target integrin	Combination therapy with	Condition	Result
NCT05085548	I	ProAgio	Cytotoxic Protein	$\alpha V\beta 3$	—	Pancreatic cancer/Solid tumors	Recruiting
NCT04389632	I	SGN-B6A	Antibody-Drug Conjugate	$\beta 6$	—	Solid tumors	Recruiting
NCT04608812	I	OS2966	First-in-class monoclonal Ab	$\beta 1$	—	High-grade Glioma	Recruiting
NCT04508179	I	7HP349	Allosteric integrin activation	In- $\alpha L\beta 2/\alpha 4\beta 1$	—	Healthy subjects	Recruiting
NCT03517176	I	CEND-1	First-in-class iRGD	$\alpha V$	Gemcitabine/Nab-Paclitaxel	Pancreatic cancer	PFS

**Table 2.** Overview of planned or ongoing clinical trials for the assessment of integrin targeting therapeutics in cancer.

A first-in-class humanized and de-immunized monoclonal antibody, OS2966, that targets the  $\beta 1$  integrin subunit is tested in patients with high-grade glioma<sup>79</sup>(NCT04608812). Considering that OS2966 targets the entire family of  $\beta 1$  containing integrins, toxicity may be an issue. Interestingly, this trial will make use of real time imaging. By adding gadolinium contrast to the OS2966 antibody, therapeutic distribution can be visualized using MRI. The additional collection of tissue specimens planned before and after treatment will provide better knowledge on the presence of any predictive biomarkers. In October 2021 a phase I trial finished, in which the safety, tolerability and pharmacokinetics (PK) of the allosteric integrin activator 7HP349 was studied in healthy male subjects (NCT04508179). Interestingly, in contrast to most integrin targeting therapeutics, this small molecule is designed to enhance integrin activity. Binding of 7HP349 should cause the activation of the  $\alpha L\beta 2$  and  $\alpha 4\beta 1$  integrins on immune cells, thereby enhancing an immune response. Results of this study remain to be published.

### Why have integrin targeted therapeutics failed to achieve clinical efficacy thus far?

Despite promising preclinical *in vitro* and *in vivo* results that indicate that integrins can be targeted with good efficacy alone, or in combination with radio-, chemo-, or immune therapies, clinical results thus far do not seem encouraging<sup>6,46,80,81</sup>. As with all experimental therapies, recruitment of sufficient numbers of patients fitting the

trial design is a challenge. As described above, for one trial this has led to early termination. In addition, testing is often done in the context of advanced disease stages and in cases where earlier therapies have failed. Patients enrolled in the clinical trials described in Table 1 typically have extensive treatment history with the exception of cilengitide that was explored in newly diagnosed glioblastoma patients. This may well explain the discrepancy between clinical trials and results obtained in more acute preclinical models. There are several other factors that may have negatively impacted the clinical testing of anti-integrin therapeutics in cancer. These include variable integrin expression in tumors, redundancy in integrin function, the fact that integrins can have very different roles at distinct disease stages and sequestering of therapeutics by integrin-containing tumor-derived extracellular vesicles (TEVs) (Figure 1).

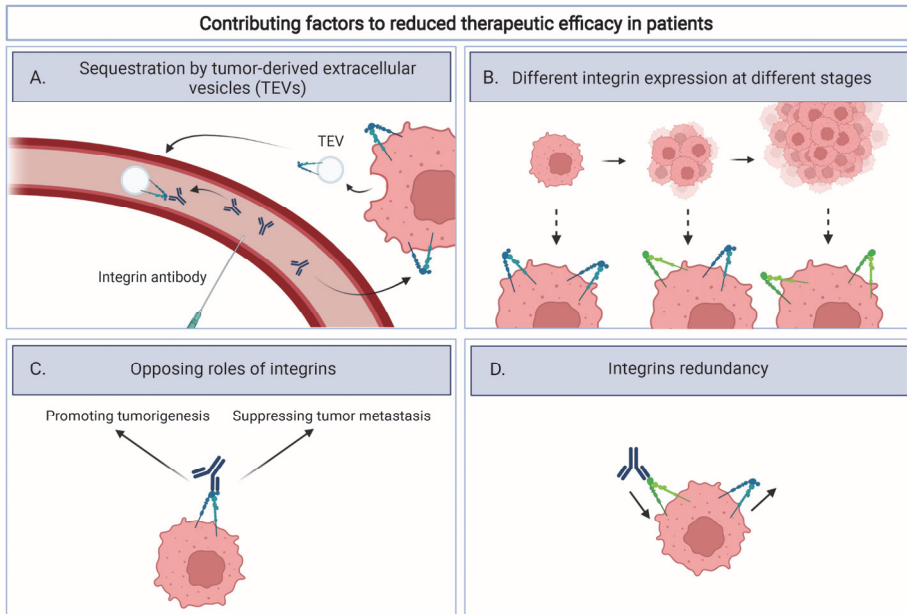
### *Variable Integrin Expression and Poor Pharmacology*

Thus far, antibodies have been the major type of anti-integrin therapeutics tested in clinical trials (Table 1). The exquisitely high specificity and corresponding low toxicity of these antibodies are most likely responsible for this high prevalence. A major limitation is a lack of knowledge with respect to expression of the target integrin in the tumor of the patient. Prior treatments may have affected integrin expression patterns in the tumor tissue. In addition, data on antibody pharmacology is generally lacking in the clinical studies. It is well known that targeting of therapeutics to the tumor tissue can be difficult due to poor vascularization<sup>82</sup> and this may be a significant problem for the relatively large antibody drugs. Hence, it is important to determine expression of the target integrin and establish actual reach of the integrin-targeting antibodies to the tumor tissue to relate these aspects to response rates in individual patients.

### *Redundancy and Different Roles of Integrins at Distinct Disease Stages*

Many integrins show overlap in their ligand binding spectrum. I.e., key ECM proteins present in cancer tissues such as fibronectin, laminins and collagens can be recognized by more than one integrin<sup>15</sup>. Hence, the effect of blocking one integrin may be compensated for by another integrin binding the same ligand. Patients entering experimental trials often present with a mix of primary and metastatic lesions at different stages. Integrin expression has been observed to differ between primary and metastatic lesions indicating that therapies may affect one but not the other stage. e.g., expression of integrin  $\alpha 2 \beta 1$  was shown to promote tumor growth of a

breast cancer cell line whereas  $\alpha 2 \beta 1$  expression was attenuated once the breast cancer cells colonized the bone<sup>22</sup>. In fact, integrins have been shown in some cases to have opposing roles at different stages and repress rather than support disease progression and metastasis. While depletion of  $\beta 1$  integrins led to reduced outgrowth of primary tumors, it enhanced metastatic capacity in an orthotopic model using triple negative breast cancer cells<sup>21</sup>. Deletion of  $\beta 1$  integrins also increased prostate cancer progression in a genetic mouse model<sup>20</sup>. Likewise, specific deletion of one of the  $\beta 1$  integrins,  $\alpha 2 \beta 1$ , was demonstrated to inhibit tumor metastasis in mouse models for breast or prostate cancer<sup>19,22</sup>. Although similar examples are not described for the  $\alpha$  integrins targeted in clinical trials thus far, these findings suggest that therapeutic targeting of integrins may lead to complex responses in patients that may vary for individual patients.



**Figure 1.** Schematic overview illustrating four factors that could contribute to the lack of clinical efficacy for integrin targeting therapeutics. These include **(A)** sequestration by tumor-derived extracellular vesicles (TEVs): integrin therapeutics bind integrins on TEVs instead of the tumor itself; **(B)** Different integrin expression at different stages: integrin expression can change as the tumor progresses and thereby influence target availability; **(C)** Opposing roles of integrins: Integrins exert tumor promoting effects but may also drive, as yet poorly understood, metastasis suppressing signals. Inhibition of integrins could therefore potentially be disadvantageous; **(D)**. Integrins redundancy: inhibition of one integrin can be compensated by expression of other integrins.

### *Sequestration of Therapeutics by Integrin-Containing Extracellular Vesicles*

Another mechanism that may underlie failure of anti-integrin drugs involves TEVs that have been implicated in tumor angiogenesis, immune evasion, and metastasis<sup>83</sup>. Tumors produce more EVs with a different cargo composition (proteins, lipids and nucleic acids) as compared to normal tissues and these EVs can be derived from the tumor cells as well as other cell types in the tumor microenvironment. Integrins are expressed on TEVs, thus guiding their preference for organ colonization<sup>84</sup>. As integrin expressing TEVs are released by various cancer types they may represent a common obstacle by sequestering integrin-targeting antibodies or peptides before these can reach their tumor target<sup>84–89</sup>. This concept has also been demonstrated for patients with inflammatory bowel disease where EVs expressing integrin  $\alpha 4\beta 7$  prevented vedolizumab from reaching  $\alpha 4\beta 7$  expressed on T cells, which may affect therapeutic efficacy<sup>90</sup>.

### **Integrins as biomarkers of cancer progression**

A major challenge for some of the most aggressive tumor types is providing an accurate diagnosis and prognosis for patients suffering from cancer. Integrins may serve as biomarkers in cancer, due to their aberrant expression on tumor cells and cells in the tumor microenvironment<sup>14–18</sup>. Recent studies reinforce the idea that some integrins may serve as predictive cancer biomarkers.

#### *Integrins $\alpha v\beta 3$ , $\alpha v\beta 5$ , and $\alpha v\beta 6$*

Integrin  $\alpha v\beta 3$  expression has been extensively associated with melanoma progression from an early radial growth phase to an invasive vertical growth and metastasis<sup>15,16</sup>. Recently, differential expression of the integrins  $\alpha v\beta 3$  and  $\alpha v\beta 6$  has been observed in two subtypes of prostate cancer. Using patient derived tumor tissue and tumor bearing murine models,  $\alpha v\beta 3$  was found to be largely absent in prostate adenocarcinoma ADPrCa but significantly upregulated in the more malignant primary neuroendocrine prostatic cancer (NEPrCa) and its metastatic lesions in the lung<sup>88</sup>. Combined with previous findings on the role of  $\alpha v\beta 3$  in the differentiation of ADPrCa to the aggressive NEPrCa,  $\alpha v\beta 3$  could have potential as a biomarker in the early detection of this malignant transition in prostate cancer<sup>88,91</sup>. The expression of integrin  $\alpha v\beta 5$  has been suggested to represent a predictive biomarker for several cancer types amongst which, breast, hepatic, and gastric carcinomas<sup>92,93</sup>. Recently, elevated levels of  $\alpha v\beta 5$  have been detected in patients suffering from

either glioblastoma or colorectal carcinoma<sup>94,95</sup>. For both types of cancer, the over-expression of  $\alpha\text{v}\beta 5$  was correlated with an unfavorable overall survival<sup>94,95</sup>. Integrin  $\alpha\text{v}\beta 6$  has been shown to represent an unfavorable prognostic marker in pancreatic cancer patients<sup>96</sup>. This integrin was recently found to be a promising serum biomarker for patients with pancreatic cancer. Based on the identification of  $\alpha\text{v}\beta 6$  in serum, chronic pancreatitis (cP) patients could be distinguished from patients with pancreatic adenocarcinoma (PAC) and high serum levels of  $\alpha\text{v}\beta 6$  were associated with poor survival<sup>97</sup>. Up to now, Carbohydrate antigen CA19-9 has been the only biomarker in use for PAC, yet its sensitivity and specificity failed to meet the expectations for use as conclusive diagnostic tool<sup>98</sup>. A study with a larger patient cohort will be needed to further assess the potential of  $\alpha\text{v}\beta 6$  alone or in combination with CA19-9 as a prognostic serum biomarker for PAC.

### *Integrin $\alpha 5\beta 1$*

Metastasis in the bones is often lethal in patients with mammary tumors<sup>99,100</sup>. Therefore, finding a predictive biomarker is essential for the early recognition of potentially metastasizing tumors. Integrin  $\alpha 5\beta 1$  is known for its participation in tumor promoting processes like angiogenesis, proliferation and metastasis<sup>17,101</sup>. In early-stage breast cancer patients,  $\alpha 5\beta 1$  expression in the primary tumor was recently associated with the presence of disseminated tumor cells in bone marrow aspirates and poor metastasis-free survival<sup>102</sup>. The same study showed that  $\alpha 5$  gene silencing or pharmacological inhibition of  $\alpha 5\beta 1$  with volociximab attenuated bone colonization following intravenous injection of tumor cells in mice. Hence, stratification of breast cancer patients based on  $\alpha 5\beta 1$  expression may represent a way to exploit the potential of  $\alpha 5\beta 1$  antibodies, which have thus far not shown clinical benefit. Integrin  $\alpha 5\beta 1$  was also found to be upregulated in several gastrointestinal tumors where enhanced expression of ITGA5 corresponded with a poor prognosis<sup>103</sup>. Again, these findings warrant larger scale patient studies to explore the potential of  $\alpha 5\beta 1$  as a prognostic biomarker in solid tumors.

### **Integrin mediated drug delivery**

In the area of drug delivery, integrin  $\alpha\text{v}\beta 3$  has been extensively pursued. It represents an attractive target because of its absence from most normal tissues versus expression in tumor tissue, including tumor cells and cells in the tumor microenvironment such as endothelial cells stimulated to undergo angiogenesis<sup>104,105</sup>. Integrin binding peptide motifs such as RGD, which binds  $\alpha\text{v}\beta 3$  as well as other integrins,



have been incorporated on the surface of drug carrying vesicles<sup>106</sup>. Cyclic RGD peptides (cRGD) have gained interest in recent years given their high binding affinity for  $\alpha v \beta 3$ <sup>107</sup>.

### *Liposomal (Like) Drug Carriers*

Liposomal vesicles have been used extensively to reduce the toxicity of conventional anti-cancer therapeutics in healthy tissues<sup>108</sup>. Low treatment efficacy with this approach is caused by ineffective reach of the tumor. The introduction of RGD peptides on the surface of liposomal like vesicles has generally enhanced both drug accumulation in the tumor and anti-tumor efficacy of the drug in mouse models<sup>109–112</sup>. Additional adjustments were made to the vesicles to further improve their drug transporting characteristics (Figure 2). Sustained drug release of the liposomes was enhanced, making use of PEGylated positively charged lipids<sup>109</sup>. The cationic liposomes decorated with the cRGD peptide were then able to deliver negatively charged siRNA into melanoma cells and effectively induce cell death<sup>109</sup>. Alternatively, Gao et al. developed a double membrane vesicle (DMV), presenting not only the RGD peptide, but also lipopolysaccharides (LPS)<sup>111</sup>. The association of LPS (normally exposed in the outer membrane of Gram-negative bacteria) with immune cells facilitated the transit of the vesicles from the vasculature into the tumor microenvironment where it could target melanoma cells and deliver therapeutics. Other  $\alpha v \beta 3$  targeting liposomal like formulations have shown a promising reduction in tumor growth for lung and hepatocellular carcinoma in *in vivo* models<sup>110,112</sup>. Liposomes targeting other integrins are slowly emerging, although selective expression of these integrins in tumor tissue is less evident. Modification of the liposomal membrane with the  $\alpha 5 \beta 1$  binding peptide PR\_b, elevated the tumor specificity of the vesicle for pancreatic cancer cells<sup>113</sup>. The addition of a thermosensitive and biodegradable hydrogel in the formulation enabled sustained release of the combination treatment paclitaxel and gemcitabine and attenuated pancreatic tumor growth. Other liposomes presenting the integrin  $\alpha 2 \beta 1$  binding ligand DGEA, were used to target breast cancer and effectively reduced tumor growth *in vivo* and enhanced the overall survival of the mice<sup>114</sup>.

### *Alternative Therapy Delivery Approaches*

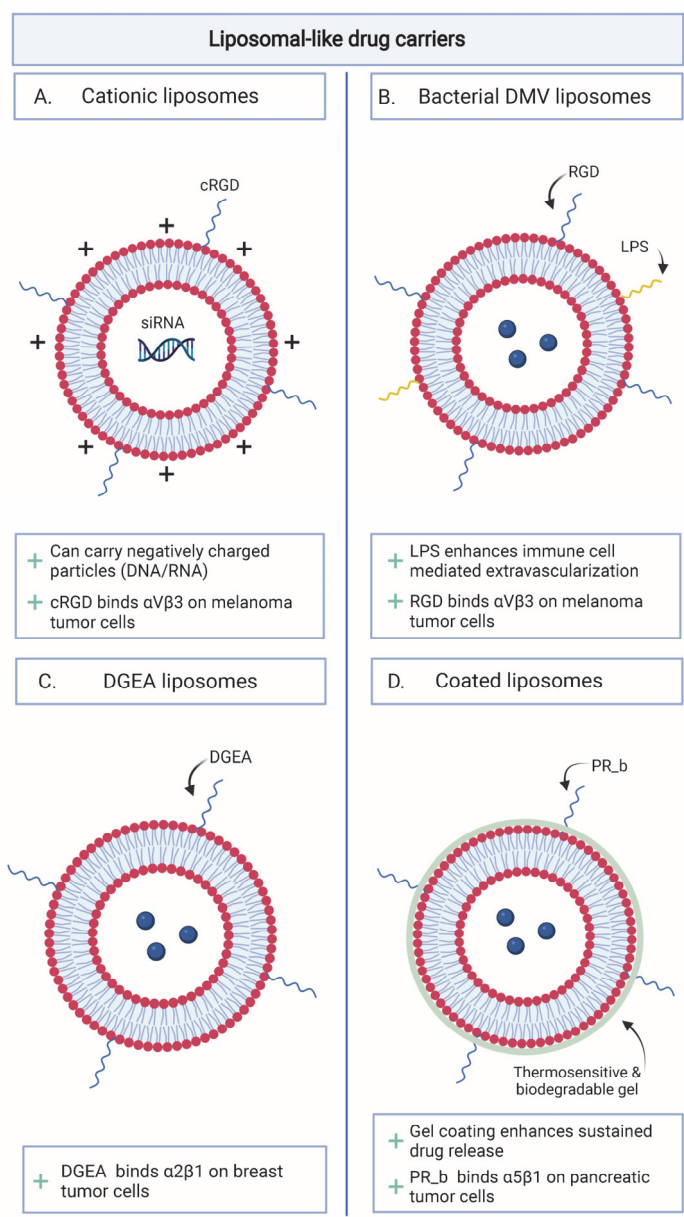
The use of integrins to direct anti-cancer therapeutics has not been restricted to their application in liposomal drug transport. Integrins may represent targets for the development of novel tumor selective immunotherapies (Figure 3A, B). In mouse models for breast cancer and head and neck squamous cell carcinoma, it was shown that  $\alpha 6 \beta 4$  is preferentially expressed on cancer stem cells (CSCs) and represents a target for immunotherapies. Vaccination with dendritic cells pulsed with  $\beta 4$  peptide or adoptive transfer of T cells incubated with  $\beta 4$ -CD3 bispecific antibodies, could induce T cell anti- tumor activity and inhibition of tumor growth and metastasis formation in the lungs of tumor bearing mouse models<sup>115</sup>. The application of covalent linking between an integrin binding peptide (mostly RGD) and an established anti-cancer therapeutic has also been explored (Figure 3D). This approach has led to reduced therapeutic- associated toxicity in healthy tissues<sup>116</sup>.

It will be interesting to compare toxicity profiles for this approach with those of liposomal encapsulations. Lastly, RGD peptides have also been incorporated in poly-dopamine (PDA) coatings to target photosensitizing agents such as gold nanostars leading to tumor specific cell death and limited adverse effects after near infrared activation of the drug<sup>117</sup>(Figure 3C).

### **Conclusions and future perspectives**

Thus far, the majority of clinical trials investigating the efficacy of therapeutics targeting integrins in cancer have failed. There are several reasons for these disappointing results, including insufficient insight into the changes in expression of integrins during cancer progression in patients and a lack of knowledge concerning the pharmacological properties and accumulation at the target site of antibodies or peptides. Analysis of these aspects would have to be included in the trial design to understand reasons for failure or success. Other difficulties include the redundancy between different integrins, the different roles that integrins have been found to play at distinct disease stages and sequestration of therapeutic antibodies or peptides by integrins present on TEVs.

We envision that 1) further understanding of these hurdles and development of approaches to combat them and 2) incorporation in the trial design of analyses of integrin expression levels and drug accumulation in the tumor tissue should provide avenues for improving therapeutic strategies targeting integrins.



**Figure 2.** Schematic overview of novel integrin targeting liposomal like drug delivery approaches. **(A)** cRGD decorated cationic liposomes; **(B)** Liposomes decorated with a combination of LPS and RGD peptides; **(C)** DGEA decorated liposomes; **(D)** Gel coated liposomes decorated with PR-b.

Integrins have been, and continue to be, explored as prognostic biomarkers in cancer, given their stage specific expression patterns. Recent studies further point to

their role in distinguishing early-stage low risk-from advanced- stage high risk, metastatic disease.

Also, their role as therapeutic targets continues to be investigated. Results thus far do not point to toxicity as a major issue for drugs targeting  $\alpha v \beta 3$  and other  $\alpha v$  integrins. It will be interesting to monitor the currently ongoing trials exploring  $\alpha 5 \beta 1$  and  $\alpha v$  integrins as targets in various cancers. The recent studies pointing to integrins as targets to attack CSCs, to activate anti-tumor immunity, or to synergize with drugs targeting immune checkpoints suggest exciting new possibilities in this field that await clinical translation. In addition, new strategies exploring integrins as targets for delivery of (liposomes containing) existing anticancer drugs are promising and may contribute to improved targeting of therapeutics and reduced toxicity. Indeed, several exciting possibilities await clinical testing and may well lead to a revisiting of integrins as therapeutic targets.

### Author Contributions

CB and KK wrote the first draft and designed figures for the manuscript. AZ and ED edited the first draft. CB, KK, AZ, and ED all read and edited subsequent drafts and read and approved the final draft.

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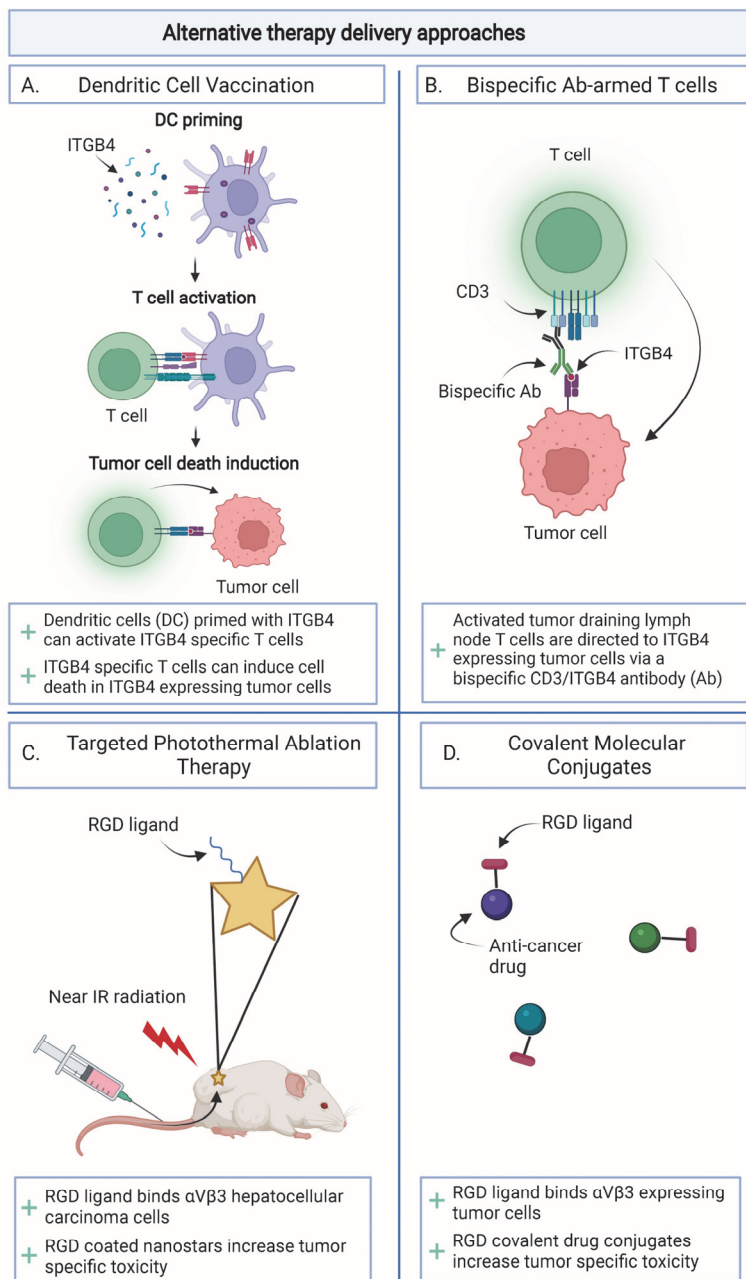
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### Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.



**Figure 3.** Schematic overview of alternative therapy delivery approaches making use of integrins. **(A)** Priming dendritic cells for vaccination; **(B)** Arming T cells with bispecific antibodies; **(C)** Targeting Photothermal Ablation Therapy; **(D)** Drug targeting through Covalent Molecular Conjugates.

### References

1. Takada, Y., Ye, X. & Simon, S. The integrins. *Genome Biol* 8, 215 (2007).
2. Barczyk, M., Carracedo, S. & Gullberg, D. Integrins. *Cell Tissue Res* 339, 269–280 (2010).
3. Kadry, Y. A. & Calderwood, D. A. Chapter 22: Structural and signaling functions of integrins. *Biochimica et Biophysica Acta (BBA) - Biomembranes* 1862, 183206 (2020).
4. Ginsberg\*, M. H. Integrin activation. *BMB Rep* 47, 655–659 (2014).
5. Calderwood, D. A. Integrin activation. *J Cell Sci* 117, 657–666 (2004).
6. Alday-Parejo, B., Stupp, R. & Rüegg, C. Are integrins still practicable targets for anti-cancer therapy? *Cancers* vol. 11 Preprint at <https://doi.org/10.3390/cancers11070978> (2019).
7. Sun, Z., Costell, M. & Fässler, R. Integrin activation by talin, kindlin and mechanical forces. *Nat Cell Biol* 21, 25–31 (2019).
8. Huveneers, S. & Danen, E. H. J. Adhesion signaling – crosstalk between integrins, Src and Rho. *J Cell Sci* 122, 1059–1069 (2009).
9. Hynes, R. O. The Extracellular Matrix: Not Just Pretty Fibrils. *Science* (1979) 326, 1216–1219 (2009).
10. Margadant, C. & Sonnenberg, A. Integrin–TGF- $\beta$  crosstalk in fibrosis, cancer and wound healing. *EMBO Rep* 11, 97–105–105 (2010).
11. Ivaska, J. & Heino, J. Cooperation between integrins and growth factor receptors in signaling and endocytosis. *Annu Rev Cell Dev Biol* 27, 291–320 (2011).
12. Brizzi, M. F., Tarone, G. & Defilippi, P. Extracellular matrix, integrins, and growth factors as tailors of the stem cell niche. *Curr Opin Cell Biol* 24, 645–651 (2012).
13. Kechagia, J. Z., Ivaska, J. & Roca-Cusachs, P. Integrins as biomechanical sensors of the microenvironment. *Nat Rev Mol Cell Biol* 20, 457–473 (2019).
14. Juliano, R. L. & Varnier, J. A. Adhesion molecules in cancer: the role of integrins. *Curr Opin Cell Biol* 5, 812–818 (1993).
15. Danen, E. H. J. Integrins: regulators of tissue function and cancer progression. *Curr Pharm Des* 11, 881–891 (2005).
16. Desgrosellier, J. S. & Cheresch, D. A. Integrins in cancer: biological implications and therapeutic opportunities. *Nat Rev Cancer* 10, 9–22 (2010).
17. Hamidi, H. & Ivaska, J. Every step of the way: integrins in cancer progression and metastasis. *Nat Rev Cancer* 18, 533–548 (2018).
18. Cooper, J. & Giancotti, F. G. Integrin Signaling in Cancer: Mechanotransduction, Stemness, Epithelial Plasticity, and Therapeutic Resistance. *Cancer Cell* 35, 347–367 (2019).
19. Ramirez, N. E. et al. The  $\alpha 2 \beta 1$  integrin is a metastasis suppressor in mouse models and human cancer. *J Clin Invest* 121, 226–237 (2011).

20. Moran-Jones, K., Ledger, A. & Naylor, M. J.  $\beta 1$  integrin deletion enhances progression of prostate cancer in the TRAMP mouse model. *Sci Rep* 2, 526 (2012).
21. Truong, H. H. et al.  $\beta 1$  Integrin Inhibition Elicits a Prometastatic Switch Through the TGF $\beta$ –miR-200–ZEB Network in E-Cadherin–Positive Triple-Negative Breast Cancer. *Sci Signal* 7, ra15–ra15 (2014).
22. Moritz, M. N. O., Merkel, A. R., Feldman, E. G., Selistre-De-araujo, H. S. & Rhoades, J. A. Biphasic  $\alpha 2\beta 1$  integrin expression in breast cancer metastasis to bone. *Int J Mol Sci* 22, (2021).
23. Friedlander, M. et al. Definition of Two Angiogenic Pathways by Distinct  $\alpha$  Integrins. *Science* (1979) 270, 1500–1502 (1995).
24. Avraamides, C. J., Garmy-Susini, B. & Varner, J. A. Integrins in angiogenesis and lymphangiogenesis. *Nat Rev Cancer* 8, 604–617 (2008).
25. Hynes, R. O. A reevaluation of integrins as regulators of angiogenesis. *Nat Med* 8, 918–921 (2002).
26. Alavi, A. S. & Cheresch, D. A. Integrins in Angiogenesis. in *Angiogenesis: An Integrative Approach From Science to Medicine* (eds. Figg, W. D. & Folkman, J.) 63–73 (Springer US, Boston, MA, 2008). doi:10.1007/978-0-387-71518-6\_6.
27. Barkan, D., Green, J. E. & Chambers, A. F. Extracellular matrix: A gatekeeper in the transition from dormancy to metastatic growth. *Eur J Cancer* 46, 1181–1188 (2010).
28. Goel, H. L. et al. Regulated splicing of the  $\alpha 6$  integrin cytoplasmic domain determines the fate of breast cancer stem cells. *Cell Rep* 7, 747–761 (2014).
29. Seguin, L., Desgrosellier, J. S., Weis, S. M. & Cheresch, D. A. Integrins and cancer: regulators of cancer stemness, metastasis, and drug resistance. *Trends Cell Biol* 25, 234–240 (2015).
30. Ata, R. & Antonescu, C. N. Integrins and cell metabolism: An intimate relationship impacting cancer. *International Journal of Molecular Sciences* vol. 18 Preprint at <https://doi.org/10.3390/ijms18010189> (2017).
31. Ji, Q. et al. Primary tumors release ITGBL1-rich extracellular vesicles to promote distal metastatic tumor growth through fibroblast-niche formation. *Nat Commun* 11, 1211 (2020).
32. Park, S.-Y. & Nam, J.-S. The force awakens: metastatic dormant cancer cells. *Exp Mol Med* 52, 569–581 (2020).
33. Winkler, J., Abisoye-Ogunniyan, A., Metcalf, K. J. & Werb, Z. Concepts of extracellular matrix remodelling in tumour progression and metastasis. *Nat Commun* 11, 5120 (2020).

34. Coban, B., Bergonzini, C., Zweemer, A. J. M. & Danen, E. H. J. Metastasis: crosstalk between tissue mechanics and tumour cell plasticity. *Br J Cancer* 124, 49–57 (2021).
35. Bagati, A. et al. Integrin  $\alpha\beta6$ –TGF $\beta$ –SOX4 Pathway Drives Immune Evasion in Triple-Negative Breast Cancer. *Cancer Cell* 39, 54–67.e9 (2021).
36. Dodagatta-Marri, E. et al. Integrin  $\alpha\beta8$  on T cells suppresses anti-tumor immunity in multiple models and is a promising target for tumor immunotherapy. *Cell Rep* 36, (2021).
37. Mohanty, A. et al. FAK-targeted and combination therapies for the treatment of cancer: an overview of phase I and II clinical trials. *Expert Opin Investig Drugs* 29, 399–409 (2020).
38. Dawson, J. C., Serrels, A., Stupack, D. G., Schlaepfer, D. D. & Frame, M. C. Targeting FAK in anticancer combination therapies. *Nat Rev Cancer* 21, 313–324 (2021).
39. Araujo, J. C. et al. Dasatinib combined with docetaxel for castration-resistant prostate cancer. *Cancer* 118, 63–71 (2012).
40. Shen, S. et al. Effect of Dasatinib vs Imatinib in the Treatment of Pediatric Philadelphia Chromosome–Positive Acute Lymphoblastic Leukemia: A Randomized Clinical Trial. *JAMA Oncol* 6, 358–366 (2020).
41. Lassman, A. B. et al. Phase 2 trial of dasatinib in target-selected patients with recurrent glioblastoma (RTOG 0627). *Neuro Oncol* 17, 992–998 (2015).
42. Kalinsky, K. et al. A phase 2 trial of dasatinib in patients with locally advanced or stage IV mucosal, acral, or vulvovaginal melanoma: A trial of the ECOG-ACRIN Cancer Research Group (E2607). *Cancer* 123, 2688–2697 (2017).
43. Martellucci, S. et al. Src family kinases as therapeutic targets in advanced solid tumors: What we have learned so far. *Cancers* vol. 12 Preprint at <https://doi.org/10.3390/cancers12061448> (2020).
44. Cabodi, S., del Pilar Camacho-Leal, M., Di Stefano, P. & Defilippi, P. Integrin signalling adaptors: not only figurants in the cancer story. *Nat Rev Cancer* 10, 858–870 (2010).
45. Bachmann, M., Kukkurainen, S., Hytönen, V. P. & Wehrle-Haller, B. Cell Adhesion by Integrins. *Physiol Rev* 99, 1655–1699 (2019).
46. Li, M. et al. Integrins as attractive targets for cancer therapeutics. *Acta Pharm Sin B* 11, 2726–2737 (2021).
47. Ley, K., Rivera-Nieves, J., Sandborn, W. J. & Shattil, S. Integrin-based therapeutics: biological basis, clinical use and new drugs. *Nat Rev Drug Discov* 15, 173–183 (2016).
48. Mitjans, F. et al. In vivo therapy of malignant melanoma by means of antagonists of  $\alpha\text{v}$  integrins. *Int J Cancer* 87, 716–723 (2000).



49. Trikha, M. et al. CNTO 95, a fully human monoclonal antibody that inhibits  $\alpha$ v integrins, has antitumor and antiangiogenic activity in vivo. *Int J Cancer* 110, 326–335 (2004).
50. Khalili, P. et al. A non-RGD-based integrin binding peptide (ATN-161) blocks breast cancer growth and metastasis in vivo. *Mol Cancer Ther* 5, 2271–2280 (2006).
51. Danen, E. H. J. Integrin Signaling as a Cancer Drug Target. *Int Sch Res Notices* 2013, 135164 (2013).
52. Hussain, M. et al. Differential Effect on Bone Lesions of Targeting Integrins: Randomized Phase II Trial of Abituzumab in Patients with Metastatic Castration-Resistant Prostate Cancer. *Clinical Cancer Research* 22, 3192–3200 (2016).
53. Élez, E. et al. Abituzumab combined with cetuximab plus irinotecan versus cetuximab plus irinotecan alone for patients with KRAS wild-type metastatic colorectal cancer: the randomised phase I/II POSEIDON trial. *Annals of Oncology* 26, 132–140 (2015).
54. Laeufle, R. et al. Patient selection for targeting integrin with abituzumab in patients with metastatic colorectal cancer (mCRC): A retrospective analysis of the randomized phase I/II Poseidon study. *Annals of Oncology* 29, viii164 (2018).
55. O'Day, S. et al. A randomised, phase II study of intetumumab, an anti- $\alpha$ v-integrin mAb, alone and with dacarbazine in stage IV melanoma. *Br J Cancer* 105, 346–352 (2011).
56. Heidenreich, A. et al. A randomized, double-blind, multicenter, phase 2 study of a human monoclonal antibody to human  $\alpha$ v integrins (intetumumab) in combination with docetaxel and prednisone for the first-line treatment of patients with metastatic castration-resistant prostate cancer. *Annals of Oncology* 24, 329–336 (2013).
57. Posey, J. A. et al. A Pilot Trial of Vitaxin, A Humanized Anti-Vitronectin Receptor (anti  $\alpha$ v $\beta$ 3) Antibody in Patients with Metastatic Cancer. *Cancer Biother Radiopharm* 16, 125–132 (2001).
58. Hersey, P. et al. A randomized phase 2 study of etaracizumab, a monoclonal antibody against integrin  $\alpha$ v $\beta$ 3,  $\pm$  dacarbazine in patients with stage IV metastatic melanoma. *Cancer* 116, 1526–1534 (2010).
59. Alva, A. et al. Phase II study of Cilengitide (EMD 121974, NSC 707544) in patients with non-metastatic castration resistant prostate cancer, NCI-6735. A study by the DOD/PCF prostate cancer clinical trials consortium. *Invest New Drugs* 30, 749–757 (2012).
60. Vermorken, J. B. et al. Cisplatin, 5-fluorouracil, and cetuximab (PFE) with or without cilengitide in recurrent/metastatic squamous cell carcinoma of the

- head and neck: results of the randomized phase I/II ADVANTAGE trial (phase II part). *Annals of Oncology* 25, 682–688 (2014).
61. Vansteenkiste, J. et al. Cilengitide combined with cetuximab and platinum-based chemotherapy as first-line treatment in advanced non-small-cell lung cancer (NSCLC) patients: results of an open-label, randomized, controlled phase II study (CERTO). *Annals of Oncology* 26, 1734–1740 (2015).
  62. Stupp, R. et al. Cilengitide combined with standard treatment for patients with newly diagnosed glioblastoma with methylated MGMT promoter (CENTRIC EORTC 26071-22072 study): a multicentre, randomised, open-label, phase 3 trial. *Lancet Oncol* 15, 1100–1108 (2014).
  63. Cianfrocca, M. E. et al. Phase 1 trial of the antiangiogenic peptide ATN-161 (Ac-PHSCN-NH<sub>2</sub>), a beta integrin antagonist, in patients with solid tumours. *Br J Cancer* 94, 1621–1626 (2006).
  64. Evans, T. et al. Final results from cohort 1 of a phase II study of volociximab, an anti- $\alpha 5\beta 1$  integrin antibody, in combination with gemcitabine (GEM) in patients (pts) with metastatic pancreatic cancer (MPC). *Journal of Clinical Oncology* 25, 4549 (2007).
  65. Figlin, R. A., Kondagunta, G. V., Yazji, S., Motzer, R. J. & Bukowski, R. M. Phase II study of volociximab (M200), an  $\alpha 5\beta 1$  anti-integrin antibody in refractory metastatic clear cell renal cell cancer (RCC). *Journal of Clinical Oncology* 24, 4535 (2006).
  66. Vergote, I. B. et al. Phase II study comparing volociximab (an antiangiogenic antibody) and pegylated liposomal doxorubicin (PLD) with PLD alone in recurrent ovarian or primary peritoneal cancer. *Journal of Clinical Oncology* 27, 5560 (2009).
  67. Barton, J. A multicenter phase II study of volociximab in patients with relapsed metastatic melanoma. *Journal of Clinical Oncology* 26, 9051 (2008).
  68. Bell-McGuinn, K. M. et al. A phase II, single-arm study of the anti- $\alpha 5\beta 1$  integrin antibody volociximab as monotherapy in patients with platinum-resistant advanced epithelial ovarian or primary peritoneal cancer. *Gynecol Oncol* 121, 273–279 (2011).
  69. Besse, B. et al. Phase Ib safety and pharmacokinetic study of volociximab, an anti- $\alpha 5\beta 1$  integrin antibody, in combination with carboplatin and paclitaxel in advanced non-small-cell lung cancer. *Annals of Oncology* 24, 90–96 (2013).
  70. Rudick, R., Polman, C., Clifford, D., Miller, D. & Steinman, L. Natalizumab: Bench to Bedside and Beyond. *JAMA Neurol* 70, 172–182 (2013).
  71. McLean, L. P. & Cross, R. K. Integrin antagonists as potential therapeutic options for the treatment of Crohn's disease. *Expert Opin Investig Drugs* 25, 263–273 (2016).

72. Yu, Y., Schürpf, T. & Springer, T. A. How Natalizumab Binds and Antagonizes  $\alpha 4$  Integrins. *Journal of Biological Chemistry* 288, 32314–32325 (2013).
73. Slack, R. J., Macdonald, S. J. F., Roper, J. A., Jenkins, R. G. & Hatley, R. J. D. Emerging therapeutic opportunities for integrin inhibitors. *Nat Rev Drug Discov* 21, 60–78 (2022).
74. Stupack, D. G., Puente, X. S., Boutsaboualoy, S., Storgard, C. M. & Cheresch, D. A. Apoptosis of adherent cells by recruitment of caspase-8 to unligated integrins. *Journal of Cell Biology* 155, 459–470 (2001).
75. Turaga, R. C. et al. Rational design of a protein that binds integrin  $\alpha \nu \beta 3$  outside the ligand binding site. *Nat Commun* 7, 11675 (2016).
76. Patnaik, A. et al. A phase 1 study of SGN-B6A, an antibody-drug conjugate targeting integrin beta-6, in patients with advanced solid tumors (SGNB6A-001, Trial in Progress). *Journal of Clinical Oncology* 39, TPS3144–TPS3144 (2021).
77. Dean, A. et al. 1528P Phase I trial of the first-in-class agent CEND-1 in combination with gemcitabine and nab-paclitaxel in patients with metastatic pancreatic cancer. *Annals of Oncology* 31, S941 (2020).
78. Dean, A. P., Mulligan, F., Kelly, G., Shaughnessy, E. & Wilson, K. Updated single institution outcome data from the first-in-human CEND-1 trial in metastatic pancreatic cancer. *Journal of Clinical Oncology* 39, e16274–e16274 (2021).
79. Nwagwu, C. D., Immidiseti, A. V., Bukanowska, G., Vogelbaum, M. A. & Carbonell, A. M. Convection-enhanced delivery of a first-in-class anti- $\beta 1$  integrin antibody for the treatment of high-grade glioma utilizing real-time imaging. *Pharmaceutics* 13, 1–15 (2021).
80. Goodman, S. L. & Picard, M. Integrins as therapeutic targets. *Trends Pharmacol Sci* 33, 405–412 (2012).
81. Raab-Westphal, S., Marshall, J. F. & Goodman, S. L. Integrins as therapeutic targets: Successes and cancers. *Cancers* vol. 9 Preprint at <https://doi.org/10.3390/cancers9090110> (2017).
82. Carmeliet, P. & Jain, R. K. Principles and mechanisms of vessel normalization for cancer and other angiogenic diseases. *Nat Rev Drug Discov* 10, 417–427 (2011).
83. Becker, A. et al. Extracellular Vesicles in Cancer: Cell-to-Cell Mediators of Metastasis. *Cancer Cell* 30, 836–848 (2016).
84. Hoshino, A. et al. Tumour exosome integrins determine organotropic metastasis. *Nature* 527, 329–335 (2015).
85. Fedele, C., Singh, A., Zerlanko, B. J., Iozzo, R. V & Languino, L. R. The  $\alpha \nu \beta 6$  Integrin Is Transferred Intercellularly via Exosomes. *Journal of Biological Chemistry* 290, 4545–4551 (2015).

86. Singh, A. et al. Exosome-mediated Transfer of  $\alpha v \beta 3$  Integrin from Tumorigenic to Nontumorigenic Cells Promotes a Migratory Phenotype. *Molecular Cancer Research* 14, 1136–1146 (2016).
87. Carney, R. P. et al. Targeting Tumor-Associated Exosomes with Integrin-Binding Peptides. *Adv Biosyst* 1, 1600038 (2017).
88. Quaglia, F. et al. Differential expression of  $\alpha v \beta 3$  and  $\alpha v \beta 6$  integrins in prostate cancer progression. *PLoS One* 16, (2021).
89. Li, X. et al. The exosomal integrin  $\alpha 5 \beta 1$ /AEP complex derived from epithelial ovarian cancer cells promotes peritoneal metastasis through regulating mesothelial cell proliferation and migration. *Cellular Oncology* 43, 263–277 (2020).
90. Domenis, R. et al. Circulating exosomes express  $\alpha 4 \beta 7$  integrin and compete with CD4+ T cells for the binding to Vedolizumab. *PLoS One* 15, e0242342- (2020).
91. Quaglia, F. et al. Small extracellular vesicles modulated by  $\alpha v \beta 3$  integrin induce neuroendocrine differentiation in recipient cancer cells. *J Extracell Vesicles* 9, 1761072 (2020).
92. Bianchi-Smiraglia, A., Paesante, S. & Bakin, A. V. Integrin  $\beta 5$  contributes to the tumorigenic potential of breast cancer cells through the Src-FAK and MEK-ERK signaling pathways. *Oncogene* 32, 3049–3058 (2013).
93. Li, Q. et al. Integrin  $\beta 5$  upregulated by HER2 in gastric cancer: a promising biomarker for liver metastasis. *Ann Transl Med* 8, 451–451 (2020).
94. Zhang, L. et al. Integrin Beta 5 Is a Prognostic Biomarker and Potential Therapeutic Target in Glioblastoma. *Front Oncol* Volume 9-2019, (2019).
95. Shi, W. et al. Integrin  $\beta 5$  enhances the malignancy of human colorectal cancer by increasing the TGF- $\beta$  signaling. *Anticancer Drugs* 32, (2021).
96. Li, Z. et al. Integrin  $\beta 6$  acts as an unfavorable prognostic indicator and promotes cellular malignant behaviors via ERK-ETS1 pathway in pancreatic ductal adenocarcinoma (PDAC). *Tumor Biology* 37, 5117–5131 (2016).
97. Lenggenhager, D. et al.  $\beta 6$ -Integrin Serves as a Potential Serum Marker for Diagnosis and Prognosis of Pancreatic Adenocarcinoma. *Clin Transl Gastroenterol* 12, (2021).
98. Goonetilleke, K. S. & Siriwardena, A. K. Systematic review of carbohydrate antigen (CA 19-9) as a biochemical marker in the diagnosis of pancreatic cancer. *European Journal of Surgical Oncology* 33, 266–270 (2007).
99. Coleman, R. E. Clinical Features of Metastatic Bone Disease and Risk of Skeletal Morbidity. *Clinical Cancer Research* 12, 6243s–6249s (2006).
100. Wang, R. et al. The Clinicopathological features and survival outcomes of patients with different metastatic sites in stage IV breast cancer. *BMC Cancer* 19, 1091 (2019).

101. Hou, J., Yan, D., Liu, Y., Huang, P. & Cui, H. The roles of integrin  $\alpha 5 \beta 1$  in human cancer. *Onco Targets Ther* 13, 13329–13344 (2020).
102. Pantano, F. et al. Integrin  $\alpha 5 \beta 1$  in human breast cancer is a mediator of bone metastasis and a therapeutic target for the treatment of osteolytic lesions. *Oncogene* 40, 1284–1299 (2021).
103. Zhu, H., Wang, G., Zhu, H. & Xu, A. ITGA5 is a prognostic biomarker and correlated with immune infiltration in gastrointestinal tumors. *BMC Cancer* 21, 269 (2021).
104. Hood, J. D. et al. Tumor Regression by Targeted Gene Delivery to the Neovasculature. *Science* (1979) 296, 2404–2407 (2002).
105. Arosio, D. & Casagrande, C. Advancement in integrin facilitated drug delivery. *Adv Drug Deliv Rev* 97, 111–143 (2016).
106. Ruoslahti, E. RGD and Other Recognition Sequences for Integrins. *Annu. Rev. Cell Dev. Biol* vol. 12 (1996).
107. Li, N., Qiu, S., Fang, Y., Wu, J. & Li, Q. Comparison of linear vs. Cyclic rgd pentapeptide interactions with integrin  $\alpha v \beta 3$  by molecular dynamics simulations. *Biology (Basel)* 10, (2021).
108. Allen, T. M. & Cullis, P. R. Liposomal drug delivery systems: From concept to clinical applications. *Adv Drug Deliv Rev* 65, 36–48 (2013).
109. Khabazian, E. et al. Cationic liposome decorated with cyclic RGD peptide for targeted delivery of anti-STAT3 siRNA to melanoma cancer cells. *J Drug Target* 30, 522–533 (2022).
110. Fu, S. et al. Integrin  $\alpha v \beta 3$ -targeted liposomal drug delivery system for enhanced lung cancer therapy. *Colloids Surf B Biointerfaces* 201, 111623 (2021).
111. Gao, J., Wang, S., Dong, X. & Wang, Z. RGD-expressed bacterial membrane-derived nanovesicles enhance cancer therapy via multiple tumorous targeting. *Theranostics* 11, 3301–3316 (2021).
112. Gong, Z. et al. Tumor acidic microenvironment-induced drug release of RGD peptide nanoparticles for cellular uptake and cancer therapy. *Colloids Surf B Biointerfaces* 202, 111673 (2021).
113. Shabana, A. M., Kambhampati, S. P., Hsia, R., Kannan, R. M. & Kokkoli, E. Thermosensitive and biodegradable hydrogel encapsulating targeted nanoparticles for the sustained co-delivery of gemcitabine and paclitaxel to pancreatic cancer cells. *Int J Pharm* 593, 120139 (2021).
114. Zhou, B. et al. Integrin  $\alpha 2 \beta 1$  Targeting DGEA-Modified Liposomal Doxorubicin Enhances Antitumor Efficacy against Breast Cancer. *Mol Pharm* 18, 2634–2646 (2021).
115. Dobson, H. E., Ruan, S., Chang, A. E., Wicha, M. S. & Li, Q. Targeting Cancer Stem Cells via Integrin B4. *Oncotarget* vol. 12 [www.oncotarget.com](http://www.oncotarget.com) (2021).

116. Cirillo, M. & Giacomini, D. Molecular delivery of cytotoxic agents via integrin activation. *Cancers (Basel)* 13, 1–25 (2021).
117. Li, Y. et al. Integrin  $\alpha\beta3$ -targeted polydopamine-coated gold nanostars for photothermal ablation therapy of hepatocellular carcinoma. *Regen Biomater* 8, rbab046 (2021).

