Transmembrane receptors are usually seen as on and off switches: when the specific ligand is bound, the receptor is on and transduces a downstream signal, whereas when the ligand is absent, the receptor is off. Over the last two decades several reports have argued for an alternative view where some receptors, depending on the context, will be active both in the presence and in the absence of ligand, being sort of onA and onB switch rather than on and off. These receptors have been named dependence receptors (DR) and they share the ability to actively trigger cell death when unbound by their respective ligands. DRs have been shown to be important guardians of tissue homeostasis. In pathological settings such as cancer, DRs are seen as tumour suppressors and a clinical trial is ongoing to assay whether these DRs can be used to provide clinical benefit by triggering cancer cell death. In this review we are reviewing this functional family of receptors and underlying their promising potential for targeted therapy against cancer.

Introduction
It has long been accepted that membrane receptors are inactive (lacking activity) entities in the absence of their ligand. However, an increasing number of observations demonstrate the existence of double-faceted receptors, activating two opposing signalling pathways depending on the presence or absence of the ligand. The receptor bound to its ligand activates a so-called ‘positive’ signalling (proliferation, survival, differentiation), whereas the unbound receptor transmits a proapoptotic signal (Fig. 1). Consequently, the expression of these receptors on the surface of cells makes the survival of these cells dependent on the presence of the ligand, hence the terminology of ‘dependence receptor’ (DR). At present, the DR family comprises about twenty members whose homology is limited to their functional duality (Fig. 2). Included in this family are netrin-1 receptors, deleted in colorectal carcinoma (DCC) [1], uncoordinated 5 homologs (UNC5Hs, UNC5H1,2,3,4 also called UNC5A,B,C,D) [2,3], the neogenin receptor [4], the low affinity neurotrophin receptor, p75 neurotrophin receptor (p75NTR) [5], receptors to the morphogen sonic hedgehog (SHH), patched-1 (PTCH-1) [6] and cell-adhesion molecule-related/downregulated by oncogenes (CDON) [7], the Semaphorin-3E receptor, Plexin D1 [8], receptors with tyrosine kinase activity, rearranged during transfection (RET) [9], tropomyosin receptor kinase A and C (TrkA and TrkC) [10,11], ephrin type A receptor 4

Abbreviations
CDON, cell-adhesion molecule-related downregulated by oncogenes; DCC, deleted in colorectal cancer; DKK-1, dickkopf-1; EphA4, ephrin type A receptor 4; IGF-1R, insuline-like growth factor 1 receptor; IR, insulin receptor; p75NTR, p75 neurotrophin receptor; PTCH-1, patched-1; RET, rearranged during transfection; SEMA-3, semaphorin-3; SHH, sonic hedgehog; TrkA and TrkC, tropomyosin receptor kinase A and C; UNC5Hs, uncoordinated 5 homologs.
(EphA4) [12], MET [13], insulin receptor (IR) and insulin-like growth factor 1 receptor (IGF-1R) [14], and anaplastic lymphoma kinase (ALK) [15], some integrins [16] and more recently Kremen-1 [17], Notch3 [18] and the GPCR receptor Latrophilin [19].

All the receptors described above, when engaged by their specific ligands, are transducing different pathways including inducing cell differentiation, migration, intercellular communication or cell survival. For most receptors, the positive signalling induced in the presence of their respective ligand is well described. While reviewing the positive signalling pathways induced by ligand is not the purpose of this review, we can cite the ability of DCC or UNC5H receptors to trigger PI3K and MAPK signalling pathway when bound to netrin-1 leading to a key role in axonal growth and orientation [20,21]. Insulin and IGF-1 activate through IR and IGF-1R receptors, many pathways such as PI3K/Akt or Ras/MAPK required for glucose uptake, glycogen synthesis or proliferation [22]. Sema3E/PlexinD1 signalling includes Ras activation and this is critical for hippocampus formation during embryonic stage [23]. Activation of Notch3 expressed on vascular smooth muscular cells (VSMC) by the Jagged1 ligand expressed on endothelial cells is necessary for VSMC maintenance and maturation via activation of a canonical pathway including Notch3 intracellular cleavage (NICD) and NICD-dependent transcription [24].

A common trait to all DR is their involvement both during embryonic development and in tumorigenesis. Thus, the expression of DR is often decreased in tumours and their expression is generally a factor of good prognosis. These observations led to the hypothesis that DRs could be tumour suppressors. DRs could indeed limit tumour progression by eliminating supernumerary cells in a limited ligand environment. Thus, DRs, via their functional duality could constitute a new natural mechanism of antitumour control. If this new antitumour mechanism has a biological significance, tumour cells capable of counteracting it will have acquired a selective advantage.

This review will be detailed in the first part the proapoptotic mechanisms of DR and in a second part, the implication of these receptors in tumour progression and their potentiality as a target for personalized anticancer therapies.

**The dependence receptor notion: a bizarre way to die**

**Importance of DR localization at the plasma membrane for cell death induction**

So far all the DRs have been shown to trigger cell death via apoptosis. Several studies have shown that DR localize in lipid rafts for the transduction of both the positive
signal or for the control of their negative signalling (proapoptotic). For example the localization in lipid rafts of DCC, UNC5A, B or C is required for induction of apoptosis. This localization is related at least in part to the palmitoylation of these receptors, which seems independent of the presence or absence of their ligand [25–27]. The accumulation of p75NTR in lipid rafts is dependent on its phosphorylation [28], whereas palmitoylation appears to reduce its association with lipid rafts [29,30]. Finally, other DR are located in rafts, including MET, integrins and PTCH-1 [16,31,32] but it is not known whether this raft localization is required for the death activity.

**Dependence receptor monomerization is key for cell death induction**

Another critical step for the induction of apoptosis seems to be the monomerization of DR. With the exception of PTCH-1 and integrins, most DRs form multimeric complexes in the presence of their ligand [33–36]. Initially described as important for positive signalling, receptor multimerization appears to play a key role in blocking the induction of apoptosis. The proapoptotic signalling of p75NTR is only induced by the monomeric form of the receptor, whereas its multimerization abrogates this induction [37]. Along the same line, the inhibition of the multimerization of DCC or UNC5B normally induced by the ligand netrin-1 is sufficient to induce apoptosis [38]. The multimerization of DRs prevents the induction of the cascade of proapoptotic events, probably by inducing structural changes in the intracellular domain. Crystallographic data showed that netrin-1 stabilizes the intracellular domain of UNC5B in a closed conformation in which the death domain (DD) interacts with the ZU5 domain, preventing apoptotic activation. Netrin-1 is unable to block apoptosis induced by a mutant UNC5B with an open constitutive conformation [39]. In its closed conformation, the UNC5B receptor interacts with the GTPase-PIKE-L resulting in the activation of the PI3 kinase signalling pathway and thus into the inhibition of the apoptotic function of UNC5B [40].

**Most DRs are cleaved by caspase in their intracellular domains**

One of the consequences of the conformational changes induced in the absence of the ligand is to render the intracellular domain of the DR susceptible to proteolytic cleavage. Indeed, one similarity shared by the majority of DRs seems to be their ability to be cleaved by caspasess in their intracellular domain. This cleavage is essential to their apoptotic function, and...
any mutation in the cleavage site abolishes cell death induction. The DCC, neogenin, PTCβ-1, ALK, EphA4, CDON, Notch3 and UNC5 receptors seems to have a unique site [1,2,4,6,15,18], whereas RET, TrkC and MET have two cleavage sites [9,10,41]. With respect to p75NTR, Lattrophilin, IR, IGF-1R and integrins, there is no evidence of cleavage by caspases. Interestingly, these cleavage sites are highly conserved in mammals, variable in other vertebrates but absent in the orthologs of lower organisms such as the nematode or Drosophila [42] suggesting that the proapoptotic function of these receptors is a relatively late event in the evolution. One may hypothesized that this late acquisition is to relate to the greater plasticity of the mammalian nervous system and the need for tune antitumour mechanisms in more complex and long-live organisms. For all DRs, binding to the ligand inhibits induction of apoptosis probably by inhibiting caspase cleavage. This hypothesis has been partially confirmed for some DR, UNC5B [43], UNC5D [44], TrkC [10], EphA4 [12] and MET [13,41]. For the other DRs, the effect of the ligand on receptor cleavage could not be studied because the cleavage products have relatively short half-lives and are difficult to detect in vivo. In addition, ectopic expression of a mutated receptor at the cleavage site is unable to induce apoptosis in the absence of the ligand [44]. On the other hand, the expression of a truncated receptor, mimicking the receptor cleaved by caspases, or the fragment released during cleavage induces apoptosis even in the presence of the ligand [1,4,6,44,45]. This observation is an indirect argument in favour of a role of the ligand in the inhibition of cleavage by caspases.

DRs as amplifiers of caspase activation

Dependence receptors are able to activate initiator or effector caspases in the absence of their ligand [46]. Activation of caspases is considered a key event in apoptosis [47]. These proteases can cleave a wide range of substrates, the consequence of which is the inactivation of survival pathways and the activation of proapoptotic mechanisms. Broad-spectrum caspase inhibitors such as zVAD-fmk block DR-induced apoptosis [42]. DCC, RET, TrkC, CDON, Notch3 and PTCβ-1 activate caspases 9 and 3 [9,10,18,36,48], p75NTR activates caspase 2 [49], whereas integrin-induced death depends on the activation of caspases 8 and 3 [16]. The molecular mechanisms involved in caspase activation by DR are beginning to be deciphered. All DRs possess in their intracellular domain, a domain required for the induction of apoptosis. The addiction/dependence domain (ADD) domain is required and often sufficient for the induction of death [50]. With the exception of DRs where caspase cleavage has not been investigated, the cleavage by caspases leads to the unmasking of ADD. Most often, the ADD is located on the part of the protein that remains anchored to the membrane. In the case of UNC5H, RET, TrkC and MET, however, it is the fragment generated by the cleavage that is proapoptotic.

In general, ADD domains are unique regions lacking structural homology with other known functional protein domains. There are, however, two notable exceptions, p75NTR and UNC5H receptors for which two regions corresponding to known functional domains are responsible for the induction of apoptosis. The first region is DD, which has homology to the DD of the TNF receptor superfamily [2,45,46,50,51]. The second region is the Chopper domain for p75NTR and the ZU5 domain for UNC5H [52]. An additional transmembrane domain with alpha helical structure, dependence-associated receptor transmembrane appears to be common to all DRs identified so far [53]. The function of this domain has not yet been clarified.

Cleavage by caspases therefore leads to the exposure of masked regions that will allow caspase amplification directly, often via the recruitment of complex protein platforms. These caspase-activating complexes vary from one DR to another and may be different from those involved in conventional apoptotic pathways. For example in the absence of netrin-1, DCC recruits (via adapter proteins to be identified) and activates caspase 9, activating in cascade the effector caspases like caspase 3. This cascade does not require the release of cytochrome c nor the formation of apoptosome (cytochrome c/apaf-1/caspase 9) as is the case in the classical mitochondrial-dependent pathway [48]. For the PTCβ-1 receptor, the domain exposed in the absence of its SHH ligand interacts with a complex comprising DRAL/ FHL2, TUCAN, a caspase 1 and 9 adapter protein containing a CARD domain and caspase 9 [36]. Interestingly, the caspase 9 activation is dependent on caspase 9 ubiquitination [54]. Similar interaction with a caspase 9 activation platform was shown for CDON [7] and more recently for Notch3 [18]. It should also be noted that the initiator caspase recruited is not always caspase 9. Stupack et al. [16] showed that the induction of apoptosis by integrins is initiated by recruitment of caspase 8.

Downstream cell death signals induced by DRs

To make the overall view even more complex, it appears that DR-induced apoptosis does not always require the formation of these activating complexes. For example the induction of apoptosis by the UNC5A, B and D has been well documented. Despite
their structural homology, these receptors seem to induce apoptosis by recruiting distinct partners. The UNC5H2/B receptor induces apoptosis in the absence of netrin-1, via the recruitment of PP2A phosphatase (by interaction with its PR65B subunit), which catalyses the dephosphorylation/activation of serine/threonine death-associated protein kinase (DAPK) present in the complex. In the presence of netrin-1, this effect is blocked by the recruitment of CIP2A, a PP2A inhibitor [55,56]. The effectors downstream of DAPK remain unknown but DAPK is known to induce cell death by p53 independent and dependent mechanisms. In addition, phosphorylation of the myosin light chain by DAPK leads to the budding of the cell membrane characteristic of apoptosis [57]. Interestingly, an interaction with DAPK is also required in apoptosis induced by Neogenin, another DR [58]. In contrast, UNC5H1/A induces apoptosis via an interaction with MAGE-D1 (formerly NRAGE) which leads to the activation of the proapoptotic kinase c-jun [59]. The UNC5H4/D receptor is cleaved by the caspases 2/3 and the released fragment is translocated into the nucleus where it interacts with E2F1 and transactivates proapoptotic genes [44]. For the TrkC receptor, in the absence of its ligand, neurotrophin-3, the double cleavage of the intracellular domain releases a proapoptotic fragment (KF-killer fragment) whose interaction with Cobra1, a potential cofactor of BRCA1, is required for induction of apoptosis [45]. TrkC KF in the presence of Cobra1 is then able to migrate to the mitochondria where there is activation of the mitochondrial pathway. A fairly similar mechanism has also been proposed for PlexinD1. PlexinD1 thus induces the activation of caspase 9 by the mitochondrial pathway [8]. In the absence of its SEMA-3E ligand, PlexinD1 interacts with the orphan nuclear receptor, NR4A1, and activates apoptosis by the mitochondrial pathway with release of cytochrome c, a known activator of caspase 9. In the presence of the SEMA-3E ligand, the PlexinD1-NR4A1 complex is dissociated, likely result of a change in receptor structure, oligomerization, and/or competitive binding of Rho GTPase Rnd2 [60–62]. It seems paradoxical that DRs are both substrates and activators of caspases. How can a receptor whose proapoptotic activity requires cleavage by caspases be an inducer of apoptosis? One possibility is that the initiation of DR cleavage is by a noncaspase protease, which would be sufficient to initiate an amplification loop by the caspases. Another possibility would be that caspases are never completely inactive, even in nonapoptotic cells, and that this residual activity is sufficient to initiate the cleavage of a nonligand-bound receptor. Interestingly, it has been observed that caspase 3 interacts with DCC downstream of the cleavage site only when netrin-1 is present or when the cleavage site is mutated [48]. Therefore, it is likely that in the presence of its ligand, DCC adopts a confirmation that prevents cleavage by caspases. In the absence of the ligand, the unmasking of the ADD domain allows the activation of caspase 9 and the amplification of caspase 3.

**Dependence receptors as guardians of tumour escape**

The ability of DRs to kill cells when the amount of ligand present in the extracellular milieu is becoming insufficient to bind all the available DRs could be seen as a mechanism to prevent cancer cells to indefinitely grow [63] (Fig. 3). To bypass this control of cell death, cancer cells may either invalidate the receptor or its proapoptotic signalling pathway, or autocrinely produce the ligand so that the receptor is always bound (Fig. 3). These two phenomena have been described and have been shown to constitute a selective advantage for tumour escape. DRs can be considered as a new family of ‘conditional’ tumour suppressors: they only exert their suppressive activity when a cell develops abnormally and in a ligand deficient territory.

**DCC, the prototypical DR, as a tumour suppressor**

Deleted in colorectal cancer, the prototypical DR, was initially identified in 1990 as frequently lost in colorectal cancers (hence its name: deleted in colorectal carcinoma) [64]. The DCC gene is in fact located on chromosome 18q, a region subject to losses of heterozygosity (LOH) frequent in colorectal cancers and more generally in a large fraction of cancers, resulting in the reduction or in the loss of complete expression of DCC [64]. These LOHs including DCC are mainly found in advanced stages of the disease, and their frequency seems to progress with tumour progression [65–68], suggesting a role for DCC not in cancer initiation but rather in their progression. In addition, restoration of DCC expression in tumour or metastatic lines reduces ganglion invasion and prevents metastatic spread of these cells to the lungs [69–72]. This initially supported the hypothesis of involvement of this tumour suppressor in the late phases of tumour progression. The loss of DCC expression has been demonstrated in many other cancers such as cancers of the stomach, prostate, endometrium, ovaries,
oesophagus, breast, testes, gallbladder or neuroblastoma and haematologic malignancies [3,73,74]. These losses of expression are not all due to LOH, and several studies have described loss of DCC expression by hypermethylation of its promoter [3,75]. In stomach cancer, hypermethylation of DCC is observed in primary tumours, but this methylation disappears in the advanced stages of the disease [76]. Unlike most other tumour suppressor genes, the first studies have suggested that somatic DCC mutations in cancers are relatively rare: only 10–15% of colon cancers had mutated DCC [20]. However, more recent studies show that DCC is in fact very frequently mutated in sun-induced melanomas [77] and a SNP in DCC has been identified in gallbladder cancer [74]. Initial doubts about the tumour suppressor function of DCC were raised by Fazeli and collaborators when they failed to show increased tumour incidence in DCC<sup>+/−</sup> mice and increased intestinal tumour progression when DCC<sup>+/−</sup> mice were backcrossed in an APC min background [20]. The doubt about the tumour suppression function of DCC was further supported by the finding of another tumour suppressor, SMAD4, in the deleted region (18q21). Nevertheless, more recent works have demonstrated that the DR function of DCC was causally implicated in its tumour suppressor activity. Mice bearing a DCC receptor mutated on the caspase cleavage site, thus unable to have a functional DCC proapoptotic activity, were generated. The absence of intestinal DCC-induced apoptosis was associated with the development of spontaneous intestinal tumours at low frequency and with increased adenocarcinoma when the DCC mutant mice were backcrossed in an APC mutant background [78]. The DCC ‘death-dead’ mutants were not only more prone to develop colorectal cancer but also more lymphoma [79]. The effect of DCC on tumorigenesis has also been studied in genetically modified mice allowing conditional invalidation of the gene. In a model of mammary carcinoma based on the somatic inactivation of p53, the invalidation of DCC leads to the appearance of metastases [80]. These results strongly support the view that DCC via its proapoptotic activity is a late tumour suppressor that limits progression and/or tumour dissemination.

The other netrin-1 receptors as tumour suppressors

UNC5H receptors are the other netrin-1 receptors and were shown to be DRs [2,3]. The expression of these
receptors (more particularly UNC5A, B and C) is often lost or greatly reduced in cancers, particularly in cancer of the ovary, breast, uterus, stomach, lung and kidney and especially in the colon cancer [81,82]. The loss of expression of UNC5H receptors in human primary tumours, as in cell lines, is mainly due to epigenetic mechanisms, such as the methylation of promoters [75,76,81,83]. In particular, the promoter region of UNC5C is hypermethylated in nearly 80% of the colorectal tumours analysed and its inactivation is correlated with the degree of aggressiveness of the pathology [83]. Expression of UNC5A in various cancer lines including colon cancer lines, reduces their ability to form colonies and induces apoptosis via caspase 3 activation [84]. Moreover, invalidation of UNC5C is associated with intestinal tumour progression in mice [83]. It should be noted that UNC5A, B and D are p53 target genes, which participate in the proapoptotic activity of p53 [43,84,85].

**Tyrosine kinase receptors as DRs and tumour suppressors**

TrkA, B and C receptors are neurotrophin receptors with preferential affinity for NGF, BDNF and NT-3 respectively. It is in the form of an oncogenic fusion protein that TrkA has been identified [86–88]. Such rearrangements have been found for TrkC in particular in congenital fibrosarcoma and acute myeloid leukaemia [89,90]. Because of their kinase activity, these receptors have been shown to play an important role in the biology of cancers, especially in the ones of neuronal or neuroendocrine origin. Surprisingly, it appeared that the three Trk receptors despite their strong homology behaved in a very dissimilar way. If TrkB is expressed in very aggressive tumours, the expression of TrkA and TrkC was on the contrary, associated with a good prognosis, at least in tumours such as neuroblastoma and medulloblastoma [91,92]. It seemed paradoxical that the strong expression of receptors with tyrosine kinase activity known to activate pro-oncogenic signalling pathways (such as the MAPK and PI3K-AKT pathways) was associated with a good prognosis. In some cases, TrkA and TrkC behave as tumour suppressors, which is consistent with the fact that they are both DRs. Accordingly, as observed for netrin-1 receptors, TrkC is underexpressed in a large fraction of colorectal cancers in humans. This decreased expression is mainly due to promoter methylation [93]. However, to date, a functional demonstration that this loss of TrkC or TrkA is promoting tumour progression still needs to be shown.

**Sonic hedgehog receptors PTCH-1 and CDON as tumour suppressors via their DR activity?**

PTCH-1, a SHH morphogen receptor, is also known as a tumour suppressor [94]. Loss of PTCH-1 expression or inactivating PTCH-1 mutations have been observed in basal cell carcinomas and medulloblastomas [95]. The classically accepted view is that its tumour suppressor activity is related to the fact that PTCH-1 represses a canonical oncogenic pathway (Smoothened-Gli). It has been shown, however, that PTCH-1 via its DR activity could reduce the tumorigenicity of cancer cells by inducing their death [6]. There is, however, currently no evidence in vivo that PTCH-1 functions as a tumour suppressor by its proapoptotic activity. This is precisely what has been demonstrated for another SHH receptor CDON. CDON recently joined the DR family [7]. High throughput sequencing has shown many false-sense mutations of CDON in human cancers (Sanger Institute Catalog for Somatic Mutations in Cancer web site, https://www.sanger.ac.uk/science/tools/cosmic). In addition, the loss of CDON expression has been observed in humans in tumours of the colon, kidney, lungs and breast [7]. Interestingly, the expression of CDON is inversely correlated with the tumour grade (according to the TNM classification) in colorectal cancers and mice mutant for CDON are more prone to develop intestinal adenocarcinoma when backcrossed with APC mutant mice [7].

**Tumour suppressive activity of other DRs**

Kremen1, one of the latest receptors to have joined the DR family, is downregulated in several cancers such as glioblastoma, neuroblastoma, breast, kidney, thyroid, colorectal or head and neck cancers, implying a putative tumour suppressor role [17,96]. Several mutations of Kremen1 in the domain responsible for the apoptotic activity induced by the receptor in absence of its ligand dickkopf-1 (DKK-1) were identified in cancer patients, supporting the view that these mutations are conferring a gain of selective advantage for the tumour survival [17].

There is also a series of evidence supporting a tumour suppressor role for other DRs. For example p75NTR is partially lost in the localized prostate tumour epithelium. This loss is inversely correlated with tumour grade (Gleason score) and total losses have been observed in metastatic prostate cancer lines [97,98]. It has been shown that EphA4 is downregulated in invasive forms of breast cancer [99], in liver and kidney cancers [100] and in metastatic melanoma
[101]. It has also been suggested that the expression of neogenin would be inversely correlated with malignancy in breast cancer [102]. Consistent with a tumour suppressor function, Notch3 is as well downregulated in breast cancer and this loss is associated with poor survival [103,104]. However, in most cases, animal models are missing in order to demonstrate a role of the proapoptotic activity of these other DRs in constraining tumour progression.

**Targeting the gain of the DRs ligand as an original targeted anticancer therapy**

If DRs can kill cancer cells in setting of limiting ligand availability, one may predict that cancer cells or more generally tumours could acquire the selective growth advantage to autocrinely produce ligands of DRs. This gain of ligand is expected to confer to the cancer cells both the ability to survive independently of ligand limitation and the amplified activation of the positive signalling below this DRs. There is a growing body of evidence indicating that this ‘gain of ligand’ has been selected in many tumours. This evidence relates more particularly on the ligand netrin-1, NT-3, SHH, Sema-3E, DKK-1 and Jagged-1. The functional evidence that a gain of ligand is associated with tumour progression was observed in mice forced to express netrin-1 in the digestive tract. The ectopic expression of netrin-1 was accompanied by a net decrease in epithelial apoptosis and these mice had a significant development of spontaneous focal hyperplasias and adenomas. Moreover, when backcrossed in an APC mutant background, the mice developed more adenocarcinoma [105]. Along the lines of the protumoral effect of this netrin-1 overexpression, netrin-1 was subsequently found upregulated in a large fraction of many other cancers such as lung cancer, prostate cancer, liver cancer, glioblastoma, neuroblastoma, metastatic breast cancer, ovarian cancer or lymphoma [79,106–108]. Netrin-1 expression was associated with a poor prognosis in patients with poorly differentiated pancreatic adenocarcinoma [109]. In breast cancer, netrin-1 is correlated with the aggressiveness of the disease and especially with its metastatic potential. An experimental silencing of netrin-1 in various cancer cell lines showed that netrin-1 is associated with cell death in vitro and with tumour growth and metastasis inhibition in mice [110,111]. In vitro cell death and tumour growth inhibition in vivo was also observed when netrin-1/receptor interfering agents were used [110–112], supporting the view that inhibition of netrin-1/DRs interaction could turn as a promising therapeutic strategy.

How netrin-1 is upregulated is mainly unknown. This is probably not through gene amplification. Of interest, overexpression of netrin-1 was also observed in a small fraction of colorectal cancers that showed activation of the NFκB transcription factor [113]. NFκB is a pivotal protein between inflammation and cancer, which participates in the regulation of immune and inflammatory responses, apoptosis and tumorigenesis [114]. Netrin-1 has then also been shown to be a direct transcriptional target of NFκB and NFκB-induced netrin-1 expression inhibits the proapoptotic activity of netrin-1 receptors [113]. Netrin-1 is also found to be overexpressed in tumours from patients with chronic inflammatory bowel diseases such as ulcerative colitis and Crohn’s disease [115] and it was shown that using netrin-1 interfering agent, one can inhibit chemically induced inflammatory colorectal cancer without affecting inflammation. This supports the view that netrin-1 induced by NFκB is promoting tumour progression probably by inhibiting tumour cell death. However, NFκB does not explain the whole overexpression of netrin-1 which is probably regulated by multiple factors. P53 is also a netrin-1 direct activator [3,112]. Of interest, it was observed that upon conventional chemotherapies, netrin-1, both in cancer cell lines and in biopsies, is massively upregulated at least in part through a p53-dependent mechanism and it was then demonstrated that combining chemotherapies with the interference with netrin-1 was associated with increased cancer cell death in vitro and increased tumour growth inhibiting effects in animal models [112]. Another way to regulate netrin-1 is probably through epigenetic regulation as demonstrated recently in breast and lung cancer [116,117].

TrkC ligand neurtrophin-3 (NT-3) is also overexpressed in a large fraction of advanced neuroblastoma and in vitro this overexpression blocks the proapoptotic activity of TrkC in human neuroblastoma lines [118]. In addition, NT-3 silencing or the inhibition of NT-3/TrkC interaction via an antibody inhibits tumour growth and metastatic spread of human neuroblastoma in xenograft models in chicken and mouse [118].

Sonic hedgehog is also upregulated in many cancers in an autocrine and paracrine manner [119,120]. However, the main view commonly accepted is that this expression is a mechanism allowing the activation of the so-called canonical pathway involving the activation of Gli transcription factors via smoothened (Smo). Nevertheless, while a potent inhibitor of the canonical pathway, the Smo antagonist, GDC-0449, has shown a beneficial effect in patients with basal cell carcinomas or medulloblastomas presenting activating
mutations of the PTCH-1-Smo-Gli pathway, this drug had no effect in other tested cancers, even though patients were stratified by their expression of SHH. The SHH signalling pathway is actually much more complex and involves other receptors (coreceptors) like CDON. We have proposed that this upregulation of SHH in cancers is not (only) activating the canonical pathway but rather could be involved in the survival of cancer cells by blocking the proapoptotic activity of CDON [7]. Preclinical models have thus suggested that the inhibition of SHH/CDON interaction by a SHH titration (SHH-TRAP) could engage in an efficient way CDON-induced tumour cell death and could potentially benefit many patients whose tumours express SHH [7].

The semaphorin Sema-3E ligand of the PlexinD1 receptor is overexpressed in a large number of cancers, and most often associated with tumour progression. The expression of Sema-3E is associated with the metastatic capacity of ovarian, colon and melanoma cancers, and is correlated with poor survival of patients with colorectal and pancreatic cancers [121–123]. Sema-3E was initially identified as a gene expressed in metastatic breast adenocarcinoma cell lines, whereas it was expressed in only 30% of non-metastatic cell lines [124,125]. Yet, the functions of Sema-3E in cancer remain unclear, especially since contradictory effects of Sema-3E have been reported.

In some models, overexpression of Sema-3E was responsible for a decrease in neo-angiogenesis and a reduction in tumour growth [122,123,126]. On the other hand, overexpression of the Sema-3E cleavage fragment by furins contributed to tumour invasion and distant metastasis formation [122,123,127,128]. The demonstration that PlexinD1 is part of the DR family has brought novel insights into the role of the Sema-3E/PlexinD1 pair in cancer. It has been shown that the autocrine production of Sema-3E increases the survival of breast cancer cell lines by inhibiting PlexinD1-activated proapoptotic signalling pathways in the absence of its ligand. Consequently, inhibiting the interaction of Sema-3E on PlexinD1 thus appears to be a potential therapeutic strategy and along this line a Sema-3E-TRAP like biologics have demonstrated, in preclinical models, tumour growth and metastasis inhibition activity [8].

The DKK-1 ligand, the ligand for Kremen-1 was found as well upregulated in an important number of cancers [129] and this overexpression is correlated with poor prognosis in cancers such as oesophageal, uterine, cervical, triple negative breast, prostate, hepatocellular or bladder [130–136]. Moreover, DKK-1 expression is correlated with cancer aggressiveness in myeloma patients [137]. Paradoxically, this overexpression was seen as counter-intuitive as upregulation of DKK-1 should be associated with the inhibition of...
Wnt pathway, a pathway that is usually seen prooncogenic. Causeret and colleagues have recently suggested that the presence of this ligand could in fact inhibit the proapoptotic function of the DR Kremen1 [17]. Of interest, even if not seen in the context of a ligand of a DR, the DKK-1 inhibition by either knock down or anti-DKK-1 antibodies approaches were considered as a potential cancer therapeutic strategy in a large panel of cancers [129,138]. Sato et al. [129] showed that interference with DKK-1 with an anti-DKK-1 antibody could limit lung tumour growth in vivo and this was accompanied by extensive cancer cell death. Furthermore, the use of an anti-DKK-1 antibody can inhibit metastasis of osteosarcoma or of hepatocellular carcinoma in xenograft mice models [138,139] or multiple myeloma dissemination in the SCID-hu mice model [140].

Of interest, the ligand gain may not only mediate the survival of cancer cells but also of stromal cells. For instance, in the tumour environment, the endothelial cells can overexpress Notch3 DR in order to trigger their own suicide; to overcome the loss of the vascular support, cancer cells are secreting in a paracrine manner the Jagged1 ligand that binds Notch3 thus limiting cell death of tumoral endothelial cells [18].

Moreover, it is also important to take into consideration that interfering with the interaction between the ligand and its respective DR may not only activate the negative ‘death’ pathway but should also inhibit the positive signalling pathway. Of relevance, some of the pathways induced by the DRs engaged by their ligands are known to promote tumorigenesis. For example neurotrophin signalling through TrkC receptor can promote brain tumorigenesis via ERK activation [141]. Also, netrin-1 is considered a proto-oncogenic protein in various cancers such as colorectal cancer, pancreatic adenocarcinoma or glioma [115,142,143]. Another DR, MET, is a well-established oncogene. MET-activating mutations have been identified in sporadic papillary renal cancer, in hepatocellular carcinoma and in gastric cancer [144]. Notch receptors have been described as oncogenic drivers as well. In particular, Notch3 activation has been associated with T-cell acute lymphoblastic leukaemia [145,146]. In this context, the proposed therapeutic strategy targeting the interaction between a DR and its ligand could therefore have a double effect: on the one hand restores a tumour suppressor activity and on the other hand inhibits a prooncogenic signal. Therefore, taking into consideration the heterogeneity of a tumour, regarding the different cellular populations but also the plasticity of cancer cells, targeting multiple DR interactions could turn as an interesting therapeutic approach (Fig. 4).

Conclusion perspective
There are accumulating evidence showing that the death activity mediated by DR observed upon ligand limitation can regulate tumour progression and that conversely interfering with ligand/DR interaction may represent on original therapeutic strategy to investigate (Fig. 3). Even though it is too early to conclude to the benefit for patients, the view of netrin-1 upregulation in a large fraction of cancer to block DR-induced cell death has led for example to the regulatory preclinical development of an antinetrin-1 antibody [117] which is now tested in a phase 1 clinical trial (https://clinicaltrials.gov/ct2/show/NCT02977195). The results of this clinical trial and the translational research performed around this trial will be decisive to demonstrate, or not, the importance of DR in cancer treatment and then consequently in biology.

Acknowledgements
The authors thank the members of Apoptosis, Cancer and Development Laboratory for their advice and contribution. The work in the authors’ laboratory is funded by institutional grants from INSERM, CNRS, University of Lyon, Centre Léon Bérard, INCA, ANR, ERC and from Ligue Contre le Cancer.

Conflict of interest
P. Mehlen discloses a potential conflict of interest as a founder and shareholder of Netris Pharma.

Author contributions
AMN and PM wrote the article together.

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