Mini-review article / *http://dx.doi.org/10.15446/rcciquifa.v49n2.89898*

EGFR and HER2 small molecules inhibitors as potential therapeutics in veterinary oncology

Juan Martín Cadena García[1,2]*, Carlos Eduardo Giraldo Murillo[1], Manuela Ramos Jaramillo[3]

[1]Universidad de Caldas, Facultad de Ciencias Agropecuarias, Departamento de Salud Animal, Calle 65 N.° 26-10, Manizales, Colombia. Código postal: 170001.

[2]Universidad de Santa Rosa de Cabal (UNISARC), Facultad de Ciencias Agropecuarias, Santa Rosa de Cabal, Colombia.

[3]Universidad de Caldas, Facultad de Ciencias Exactas y Naturales, Calle 65 No 26-10, Manizales, Colombia.

*Corresponding author E-mail address: : juan.cadena@ucaldas.edu.co

Received: 17 August 2019

Revised: 10 April 2020

Accepted: 14 May 2020

Summary

EGFR and HER2 receptors are crucial signaling molecules tyrosine kinase involved in human cancer. Aberrant signaling is associated with a variety of cancers, frequently with poor prognosis. Currently, EGFR and HER2 receptors are being targeted by small molecules, which offer a huge benefit to those patients afflicted by aggressive forms of cancer, improving their prognosis. Both human and canine cancers share molecular, biological, histopathological, and clinical similarities, including EGFR and HER2 expression in some forms of cancer. However, despite the use of one tyrosine kinase inhibitor approved to treat canine mastocytoma, canine cancers overexpressed EGFR and HER2 do not yet have targeted therapy, leading to high morbidity and mortality. Targeting EGFR and HER2 receptors in canine cancers using comparative approaches in human cancer could lead to better outcomes.

Key words: Human cancer, veterinary cancer, EGFR, HER2, inhibitors.

Resumen

Inhibidores EGFR y HER2 como tratamientos potenciales en oncología veterinaria

El receptor de factor de crecimiento epidérmico (Epidermal growth factor receptor, EGFR) y el receptor 2 del factor de crecimiento epidérmico (HER2 epidermal growth factor receptor 2) son moléculas señalizadoras cruciales pertenecientes a la familia de proteínas tirosina quinasa involucradas en el cáncer en humanos. La señalización aberrante de dichos receptores se encuentra asociada con una variedad de tumores, frecuentemente asociados a mal pronóstico. Actualmente, EGFR y HER2 son tratados específicamente a través de pequeñas moléculas inhibidoras, las cuales ofrecen un gran beneficio a aquellos pacientes que padecen formas agresivas de cáncer, y de esta manera su pronóstico mejora. Tanto el cáncer en medicina humana como veterinaria comparte similitudes moleculares, biológicas, histopatológicas y clínicas, las cuales incluyen la expresión tanto de EGFR y HER2 en algunas formas de cáncer. Sin embrago, a pesar del uso de un inhibidor tirosina quinasa aprobado para el manejo del mastocitoma canino los tumores que se caracterizan por la sobreexpresión de EGFR y HER2 aún no cuentan con un inhibidor específico, lo cual conduce a alta morbilidad y mortalidad.

Palabras clave: Cáncer humano, cáncer en veterinaria, EGFR, HER2, inhibidores.

Introduction

Cancer is the most important cause of death in dogs worldwide [1-3]. As observed in human patients EGFR and HER2 receptors, tyrosine kinase receptors that play a fundamental role in the control of fundamental transduction and signaling cellular pathways involved in cell survival, cell proliferation, angiogenesis, cell adhesion, cell motility, development, and organogenesis, their dysregulation can result in both development and progression of a variety of tumors [4, 5]. Their overexpression and amplification are associated with poor prognosis in both species, however by contrast with human medicine, small animal veterinary oncology lacks small molecule inhibitors targeting EGFR and HER2, despite the evidence of *in vitro* activity in canine cancer cell lines of some approved EGFR and HER2 small molecule inhibitors for use in humans [6, 7], in which using inhibitors can provide better prognosis. The objective of this literature research was to review and discuss the role of EGFR and HER2 in the biology of both human and veterinary cancer, and the potential benefit of using EGFR and HER2 small molecule inhibitors in veterinary practice.

EGFR AND HER2 STRUCTURE

EGFR (epidermal growth factor receptor) and HER2, belong to the ErbB subclass of tyrosine kinases receptors (RTKs), one of the most studied signaling proteins and signaling transduction pathways regulators in biology [8-11]. EGFR was the first HER receptor identified as tyrosine kinase receptors a revolutionary discovery [8]. The coding genes of these receptors are EGFR/ERBB1/HER1, ERBB2/HER2/NEU, and can be found in two different chromosomes [12]. Under normal conditions, EGFR and HER2 are expressed by epithelial, mesenchymal, neuronal, cardiac, and mammary tissues [13-15].

The structure presents the typical kinase bi-lobed folding. The N-terminal lobe contains mainly β-strands and a one α-helix, whereas the C-terminal lobe is mostly α-helical [16]. The two lobes are connected through a flexible hinge region and separated by a cleft functioning as docking site for ATP [16].

Globally, EGFR and HER2 consist of an extracellular domain, a single hydrophobic transmembrane α-helix, a juxtamembrane segment, and a catalytic domain with tyrosine kinase activity [12]. ErBb family members exist as monomers, the ligand binding to the extracellular domain results in the formation of either homo or heterodimers, a process that activates the catalytic cytoplasmic domain and the C-terminal phosphorylation initiates further downstream signaling pathways [8].

Although EGFR can HER2 is the preferred dimerization partner to all the other members, the heterodimers forming with HER1 and HER3 manifest a strong signaling activity [8, 10, 17]. The binding domains of ErbB family are stimulated by eleven polypeptide growth factor ligands distributed in 4 subgroups, which results in the formation of dimers [18]. In contrast to EGFR, HER2 receptors are unable to bind growth factors physiologically. However, HER2 can form functional homodimers under non-physiological expression, an important phenomenon in carcinogenesis [19].

EGFR AND HER2 SIGNALING PATHWAYS

EGFR and HER2 signaling pathways correspond to complex networks, their specificity and potency are determined by several factors, and however, the most important determinant is the variety of binding proteins associated with the carboxy-terminal tail from each member of the family [20]. The autophosphorylated sites are determined both by the ligand identity and by the heterodimer partner [20]. HER2 can binds to a much quantity of phosphotyrosine-binding proteins in contrast to other receptors

of the ErbB family [21]. Additionally, EGFR and HER2 heterodimers present higher affinity and broader specificity to various ligands than other heterodimeric receptor complexes in this family, and have slow rates of growth-factor dissociation, slow endocytosis and more recycle to the cell surface [22, 23]. This confers potent mutagenic signaling [24] due to the recruitment of multiple signaling pathways [10]. Ras (Ras/Raf/MEK/ERK1/2 pathway), phospholipase C (PLCγ), Shc-activated mitogen-activated protein kinase (MAPK) and STAT (signal transducer and activator of transcription) proteins, especially STAT3 and STAT5 pathway are common targets of all the family ligands and PI3K/AKT are activated by the majority of the active dimers [5, 8, 25, 26]. The activation of simultaneous signaling cascades, including the MAPK pathway, the stress-activated protein cascade, protein kinase C (PKC) and the Akt pathway results in activation of transcriptional activity in the nucleus [8]. This variety of process involves both the proto-oncogenes fos, myc and Sp1 and Egr1 a family of zinc-finger-containing transcription factors and GA-binding protein (GABP) one of the Ets family members [25].

EGFR and HER2 signaling pathways play fundamental roles in cell survival, cell proliferation, angiogenesis, cell adhesion, cell motility, development, and organogenesis [5, 25]. However, their aberrant signaling results in key events leading to cancer development and progression [18].

EGFR, HER2, AND ONCOGENESIS

EGFR and HER2 dysregulation represent a potent oncogenic trigger [12, 27, 28]. The underlying mechanisms leading to this dysregulated activity include extracellular and cytoplasmatic domains mutations, which cause increased biosynthesis and levels of ErbB proteins. These mechanisms are tumor-specific [28].

Through receptors deregulation matrix metalloproteinases (MMPs) process its ligands, facilitating autocrine activation and growth signals auto-sufficiency [1, 28]. Loss of suppressor genes functions results in EFGR and HER receptors recycling, phenomena that induces the insensibility to growth inhibition [12, 28]. PI3K and STAT signaling inhibit apoptosis, a potent mechanism regulator of cellular survival, additionally, STAT and MAPK signaling provide to cells with unlimited replicative potential [12]. Both STAT3 activation and vascular endothelial growth factor (VEGFR) transactivation results in sustained angiogenesis [29]. Finally, PLCγ, MAPK and MMP pathways are associated with invasion and metastasis [12, 30].

EGFR AND HER2 EXPRESSION IN HUMAN CANCERS

When EGFR and HER2 are deregulated, they can become potent oncogenic triggers [5, 31]. Resulting effects from aberrant EGFR and HER2 signaling pathways induce the hallmarks of cancer (self-sufficiency in growth signals, inhibitory signals insensitivity, apoptosis evasion, angiogenesis, unlimited replication, invasive potential, and metastasis) [31, 32]. Deregulated receptors induce self-ligands processing, and hence, can establish autocrine-signaling loops, which provides EGFR and HER2 the ability to generate their own growth signals [5]. Frequently, loss of regulation occurs because of degradation evasion, and gain sustained signaling. Evasion results whether from increased reutilization or reduced degradation [33, 34]. Additionally, the loss of function of tumor suppressor proteins and constitutively activated receptors [35] increase the effect of inhibitory signals insensitivity [5].

Both amplification or overexpression of EGFR are frequently observed in breast cancer, non-small lung cancer [36], colorectal, urinary bladder, pancreatic, ovarian [18], head and neck squamous cell carcinoma [37, 38], renal [39], hepatocellular carcinoma [40], stomach [41], glioma, meningioma [42, 43], glioblastoma [44], and astrocytoma [8, 20], and they are associated with progression of disease, radiotherapy resistance, and poor survival [45, 46].

On the other hand, both amplification and overexpression of HER2 has been documented in breast and stomach cancer [47], salivary ducts carcinoma [48], ovarian cancer, pancreatic, cervical, endometrium, colon, glioblastoma, head and neck, non-small cell lung cancer, hepatocellular carcinoma, urinary bladder carcinoma, and pediatric osteosarcoma in variable degrees [49-52]. Its expression has been associated with an aggressive phenotype, highly metastatic degree, and poor prognosis [52].

EGFR AND HER2 EXPRESSION IN VETERINARY ONCOLOGY

Despite the role of EGFR and HER2 is well characterized in human medicine, in veterinary medicine is still in its early stages [53]. In veterinary oncology, several studies have documented the overexpression of EGFR and HER2 and its correlation with prognosis, however, these results have been in certain degree contradictory [54], hence further research is needed [5]. In veterinary cancer, EGFR expression has been demonstrated in a variety of cancer, including head and neck squamous cell carcinoma [55], hepatocellular carcinoma [56, 57] , astrocytoma [5, 43], and glioblastoma [58]. There is an increasing body of evidence about the function of EGFR either in normal and neoplastic mammary tissue in dogs, and its potential as a pharmacological target [59].

In canine mammary tumors, one of the most common in dogs a recent study reports the positive correlation between EGFR expression by immunohistochemistry with angiogenesis, histological grade of malignancy, mitotic grade, and clinical stage in canine mammary tumors [60]. Another research evaluated EGFR immunohistochemical expression in 138 tumor specimens, and revealed overexpression in 38 tissues (42.2%), this finding was associated with age, and tumoral size, and showed a relation between overexpression and malignant behavior [61]. Based on 5 molecular phenotypes established in breast cancer according to estrogens, progesterone, and HER2 status [62], in canine mammary tumors researchers have attempted to evaluate the potential of this system [62-64]. One of these studies have revealed 45 de 241 to triple negative (18.7%), 32 (71.1%) was positive to EGFR, and 13 (28.9%) was negative, and they were associated with pathological parameters (grade III, central necrosis, lymphatic infiltration, and high mitotic index) [62]. Another recent research has quantified EGFR in canine mammary tumors using ELISA in 75 specimens of 45 affected patients and 8 controls and demonstrated a statistically significant difference between both groups and a correlation with relapse, and distant metastasis during follow-up, and both reduced global survival and disease-free time. These findings provide the rationale to the implementation to therapeutic intervention, in particular in cases with aggressive behavior [65].

In HER2, its expression has been documented in diverse types of cancer in dogs, especially in canine mammary tumors, which exists larger emphasis, there exists a correlation between overexpression and malignant behavior [61, 66]. Additionally, certain evidence in other types of cancer, such as transitional cell carcinoma in urinary bladder [7, 67], and canine osteosarcoma, has also reported [68]. Clonal aberrations have been identified in the HER2 gene in some types of cancer in dogs [69].

EGFR AND HER2 TYROSINE KINASE INHIBITORS

All small molecules EGFR and HER2 inhibitors, gefitinib (Iressa®, AstraZeneca), erlotinib (Tarceva1®, OSI Pharmaceuticals), lapatinib (Tykerb®, GlaxoSmithKline), vandetanib (Caprelsa®, AstraZeneca), afatinib (Gilotrif®, Boehringer Ingelheim), neratinib (Nerlynx®, Puma Biotechnology, Inc), and dacomitinib (Vizimpro®, Pfizer), belong to quinazoline scaffold binding and occupying adenine of adenosine triphosphate (ATP) pocket, and are one of the most largest groups of RTKs inhibitors approved [70]. The members of this group are classified in reversible belong to the first generation, and irreversible belong to the second generation. EGFR and HER2 inhibitors are further categorized into four types based on the conformation of the binding pocket and the Asp-Phe-Gly (DFG) motif [71, 72] from type I to type V [70].

First generation of EGFR and HER2 inhibitors

Lapatinib

Lapatinib distosylate monohydrate (Tykerb®, GlaxoSmithKline) an oral small molecule reversible inhibitor [73] that targets both EGFR and HER2, was approved by the FDA in March 2007 to be used in combination with capecitabine (Xeloda®; Roche) for patients with metastatic breast cancer overexpressing HER2 and who have received prior therapy including an anthracycline, a taxane and trastuzumab [14]. Lapatinib binds the kinase ATP-binding cleft of EGFR on its inactive conformation in contrast to other HER family inhibitors (erlotinib and gefitinib) that exhibit type I inhibition mechanism [70], additionally uses an allosteric pocket formed by the conformation change of the DFG motif [74]. Lapatinib binds with the inactive conformation of HER4 and HER2 due shares the same contacting residues between both [75], its EGFR, HER2, and HER4 inhibition leads to inhibition of substrate phosphorylation and blocks MAPK and PI3K/Akt and treated cells depending on tumor type can undergo either apoptosis or growth arrest [73].

Second generation of EGFR and HER2 inhibitors

Neratinib

Neratinib maleate (Nerlynx®, Puma Biotechnology, Inc), is an irreversible small molecule inhibitor initially designed to target specifically HER receptor using a model of homology for the catalytic domain [76]. Neratinib binds covalently with a cysteine residue in the adenosine triphosphate (ATP)-binding pocket of HER receptor kinases (Cys773 in EGFR and Cys805 in HER2), which is tough as a property to compete with the high concentration of ATP and provide prolongs inhibition of kinase catalytic activity [77]. It is considered as a pan-HER inhibitor because the cysteine residue required for binding is conserved in these three HER receptors [78]. Neratinib inhibits phosphorylation of MAPK [77], retinoblastoma gene product, blocks cell cycle progression, cyclin D1 expression, increase p27 levels (an inhibitor of cell cycle progression), which result in G1-S arrest and an increase in cells with sub-G1 DNA content, associated with apoptosis [77]. Nerlynx® is indicated as adjuvant treatment of patients with early stage HER2-overexpressed following adjuvant trastuzumab-based therapy [79].

Afatinib

Afatinib, an aniline-quinazoline, is an oral, potent irreversible EGFR and HER2 inhibitor oral. Quinazoline ring binds to catalytic domain as observed with

inhibitors EGFR and HER2 [80] [81] According to efficacy on in vitro and in vivo assays in lung cancer [82, 83] afatinib was approved in 2013 as Gilotrif ®, Boehringer Ingelheim Pharmaceuticals, Inc, to the therapy of non-small cell lung cancer. Its potency in vitro is better than gefitinib and lapatinib due to covalent nature [82]. Afatinib has been effective to a variety of cell lines of breast cancer (BT-474, SUM190-PT, SUM 149-PT y T47D). In fact, this finding was crucial to elucidate its action against Her3, associated with the ability to confer cellular survival to cancer cells throughout the PI3K/Akt pathway [84]. Afatinib has demonstrated synergistic activity to mTOR inhibitor rapamycin and trastuzumab [83], and longer duration of action in vitro in contrast with reversible inhibitors erlotinib, gefitinib y lapatinib [82, 85]. Nowadays the potential role of afatinib as adjuvant therapy with paclitaxel in breast cancer is investigated, however, the results have not yet released [86]. In breast cancer, afatinib has been administered because of HER2 inhibition with promising results in HER overexpressing patients with combination to first-line therapies [87]. Adverse effects documented are very similar to those observed with lapatinib (diarrhea and skin reactions).

Dacomitinib

Dacomitinib inhibits irreversibly EGFR, HER2, and HER4. Its preclinical efficacy was demonstrated only on EGFR T790M, and some HER2 cell lines [88-90]. In both phases, I and II, dacomitinib was safe and efficient in patients with non-small cell lung cancer, even compared with patients treated with erlotinib [91]. Adverse effects observed in different trials are diarrhea, dermatitis acneiform, and other types of skin toxicities, and stomatitis [91]. In 2018 was approved the first-line treatment of patients with metastatic non-small cell lung cancer (NSCLC) with epidermal growth factor receptor (EGFR) exon 19 deletion or exon 21 L858R substitution mutations.

Comparative oncology: opportunities for both species

The insights in the molecular and genomic research of cancer have improved the understanding about the similarities between humans and dogs and have led to the concept of comparative oncology, which has the objective to accelerate simultaneously the cancer drug development in both species [92]. Whole canine genome sequence and assembly released in 2005 and improved in 2014 has revealed strong similarities in genes implied in cancer development and progression [93, 94]. Additionally, it is widely accepted that both humans and dogs share certain features in cancer, including determined risk factors, such as age, hormonal, environmental influence, histological types, overexpression of a variety of biomarkers such as cellular proliferation

(Ki67, AgNor), p53 mutations, EGFR, MMPs, cyclooxygenase 2 (COX2), molecular, clinical behaviors and outcomes, however also exist significant differences [95-98].

Tyrosine kinase inhibitors in veterinary oncology

Actually, in veterinary oncology only exists one small molecule inhibitor of RTKs approved to clinical use in canine patients by FDA, toceranib phosphate (Palladia®, Pfizer Animal Health), an oral small molecule inhibitor targeting VEGFR2, platelet-derived growth factor receptor (PDGFRα) and c-kit [99]. Due Palladia® has structural similarity with Sunitinib® an oral small molecule inhibitor targeting VEGFR2, VEGFR3, PDGFRα/β, KIT, CSF1R, FLT-3, and RET, probably has an additional activity to these molecules [100]. Toceranib phosphate was initially developed as an antiangiogenic drug, however, due to its wider pharmacological profile that includes KIT and FLT-3 causes antitumor activity [101].

Its first clinical efficacy in canine cancer patients was a phase 1 clinical trial, which included 57 dogs with diverse temporal types including sarcomas, carcinomas, melanomas, myelomas, and mastocytomas. The response in 16 patients was observed: 6 with complete response and 10 with partial response and stable disease. This study documented a biological activity of 54% [99].

After its FDA approved licensed in June 2009, has been utilized as an extra label in other temporal types that failed to standard treatments, including metastatic osteosarcoma, anal sacs adenocarcinoma, thyroid carcinoma, head, and neck carcinoma, and nasal carcinoma documented clinical benefit in 63 of 85 dogs [102]. Additionally, have been documented responses in a case of [103], synergistic activity with piroxicam in transitional and squamous cell carcinoma and with vinblastin in mastocytoma [104, 105].

In combination with radiotherapy its efficacy was evaluated, was observed the objective response in 76.4%, 58.8% of canine patients achieved a complete response and 17.6% partial response, which suggest clinical benefit [106].

Imatinib (Gleevec®, Novartis) an oral small molecule inhibitor targeting Bcr/Abl, platelet-derived growth factor (PDGF) and stem cell factor (SCF) c-kit in human patients with chronic myelogenous leukemia (CML), myelodysplastic/myeloproliferative diseases, aggressive systemic mastocytosis, hypereosinophilic syndrome and/or chronic eosinophilic leukemia (CEL), dermatofibrosarcoma protuberans, and malignant gastrointestinal stromal tumors (GIST), demonstrated tolerance in canine patients [107-109].

An interesting *in vitro* study with Gefitinib (Iressa1®, AstraZeneca), an oral small molecule inhibitor targeting EGFR, conducted in a cellular line of CMT (REM134) demonstrated a favorable effect in cellular proliferation, migration. Additionally, other small molecule inhibitors such as AG825, which targeting HER2 and GW583340, which targeting EGFR and ERBB2, have documented activity, with the potential use in veterinary oncology clinical trials [*6*].

Conclusions

As documented in human cancer, in canine patients the aberrant expression of EGFR and HER2 receptors constitute a potent oncogenic trigger, however in canine patients still clearly unresolved the expression patterns and the precise molecular structure, which limit its inclusion in validated clinical trials. Despite the increasing evidence of the biological activity of EGFR and HER2 small molecule inhibitors using in human medicine in canine cancer cell lines, their clinical use not have been yet received special attention, in contrast to toceranib phosphate and masitinib mesylate in veterinary oncology. Actually, there is an increasing interest in developing targeted therapies in veterinary oncology. The *in vitro* efficacy documented regarding some small molecule tyrosine kinase inhibitors, can constitute an interesting starting point to initiate the investigation of therapeutic potential to target EGFR and HER2 in veterinary cancer and then improve the clinical outcomes, survival rates, and optimize health quality of life of these patients. Veterinary cancer high prevalence requires targeted therapies, and the routinely uses of EGFR and HER2 small molecules inhibitors represent an excellent opportunity to address this worldwide problem that affects dogs.

Disclosure statement

No potential conflict of interest was reported by the authors.

References

1. V.J. Adams, K.M. Evans, J. Sampson, J.L.N. Wood, Methods and mortality results of a health survey of purebred dogs in the UK, *J. Small Anim. Pract.*, **51**(10), 512-524 (2010).

2. J.M. Fleming, K.E. Creevy, Mortality in North American dogs from 1984 to 2004: An investigation into age-, size-, and breed-related causes of death, *J. Vet. Intern. Med.*, **25**(2), 187-198 (2011).

3. A. Egenvall, A. Nødtvedt, J. Häggström, B. Ström-Holst, L. Möller, B.N. Bonnett, Mortality of life-insured Swedish cats during 1999 –2006: Age, breed, sex, and diagnosis, *J. Vet. Intern. Med.*, **23**(6), 1175-1183 (2009).

4. P. Blume-Jensen, T. Hunter, Oncogenic kinase signalling, *Nature*, **411**, 355-365 (2001).

5. G.T. Bergkvist, D.A. Yool, Epidermal growth factor receptor as a therapeutic target in veterinary oncology, *Vet. Comp. Oncol.*, **9**(2), 81-94 (2011).

6. M.E. Gray, S. Lee, A.L. Mcdowell, *et al.*, Dual targeting of EGFR and ERBB2 pathways produces a synergistic effect on cancer cell proliferation and migration *in vitro*, *Vet. Comp. Oncol.*, **15**(3), 890-909 (2017).

7. K. Sakai, S. Maeda, K. Saeki, *et al.*, Anti-tumour effect of lapatinib in canine transitional cell carcinoma cell lines, *Vet. Comp. Oncol.*, **16**(4), 642-649 (2018).

8. R. Roskoski, Jr., The ErbB/HER family of protein-tyrosine kinases and cancer, *Pharmacol. Res.*, **79**, 34-74 (2014).

9. M.A. Lemmon, J. Schlessinger, Cell signaling by receptor tyrosine kinases, *Cell*, **141**(7), 1117-1134 (2010).

10. A. Citri, Y. Yarden, EGF – ERBB signalling : towards the systems level, *Nat. Rev. Mol. Cell Biol.*, **7**, 505-516 (2006).

11. P. Littlefield, L. Liu, V. Mysore, Y. Shan, D.E. Shaw, N. Jura, Structural analysis of the EGFR /HER3 heterodimer reveals the molecular basis for activating HER3 mutations, *Sci. Signal.*, **7**(354), ra114 (2014).

12. G.T. Bergkvist, D.A. Yool, Epidermal growth factor receptor as a therapeutic target in veterinary oncology, *Vet. Comp. Oncol*., **9**(2), 81-94 (2011).

13. N.E. Hynes, H.A. Lane, ERBB receptors and cancer: the complexity of targeted inhibitors, *Nat. Rev. Cancer*, **5**(5), 341-354 (2005).

14. B. Moy, P. Kirkpatrick, S. Kar, P. Goss, Lapatinib, *Nat. Rev. Drug Discov.*, **6**(6), 431-432 (2007).

15. R. Nahta, G.N. Hortobagyi, F.J. Esteva, Growth factor receptors in breast cancer: potential for therapeutic intervention, *Oncologist*, **8**(1), 5-17 (2003).

16. K. Aertgeerts, R. Skene, J. Yano, *et al.*, Structural analysis of the mechanism of inhibition and allosteric activation of the kinase domain of HER2 protein, *J. Biol. Chem.*, **286**(21), 18756-18765 (2011).

17. E. Tzahar, H. Waterman, X. Chen, *et al.*, A hierarchical network of interreceptor interactions determines signal transduction by Neu differentiation factor/neuregulin and epidermal growth factor, *Mol. Cell. Biol.*, **16**(10), 5276-5287 (1996).

18. R. Roskoski, Jr., The ErbB/HER receptor protein-tyrosine kinases and cancer, *Biochem. Biophys. Res. Commun.*, **319**(1), 1-11 (2004).

19. R. Ghosh R, A. Narasanna S.E. Wang, *et al.*, Trastuzumab has preferential activity against breast cancers driven by HER2 homodimers, *Cancer Res.*, **71**(5), 1871-1882 (2011).

20. Y. Yarden, M.X. Sliwkowski, Untangling the ErbB signalling network, *Nat. Rev. Mol. Cell. Biol.*, **2**(2), 127-137 (2001).

21. R.B. Jones, A. Gordus, J.A. Krall, G. Macbeath, A quantitative protein interaction network for the ErbB receptors using protein microarrays, *Nature*, **439**, 168-174 (2006).

22. A.E. Lenferink, R. Pinkas-Kramarski, M.L. van de Poll, *et al.*, Differential endocytic routing of homo- and hetero-dimeric ErbB tyrosine kinases confers signaling superiority to receptor heterodimers, *EMBO J.*, **17**(12), 3385-3397 (1998).

23. J. Baulida, M.H. Kraus, M. Alimandi, P. Paolo, D. Fiore, G. Carpenter, All ErbB receptors other than the epidermal growth factor receptor are endocytosis impaired (*), *J. Biol. Chem.*, **271**(9), 5251-5257 (1996).

24. R. Pinkas-kramarski, L. Soussan, H. Waterman, *et al.*, Diversification of Neu differentiation factor and epidermal growth factor signaling by combinatorial receptor interactions, *EMBO J.*, **15**(10), 2452-2467 (1996).

25. G. Lurje, H.J. Lenz, EGFR signaling and drug discovery, *Oncology*, **77**(6), 400-410 (2009).

26. T. Bowman, R. Garcia, J. Turkson, R. Jove, STATs in oncogenesis, *Oncogene*, **19**(21), 2474-2488 (2000).

27. S.P. Soltoff, L.C. Cantley, p120cbl is a cytosolic adapter protein that associates with phosphoinositide 3-kinase in response to epidermal growth factor in PC12 and other cells, *J. Biol. Chem.*, **271**(1), 563-567 (1996).

28. R. Zandi, A.B. Larsen, P. Andersen, M.T. Stockhausen, H.S. Poulsen, Mechanisms for oncogenic activation of the epidermal growth factor receptor, *Cell Signal*, **19**(10), 2013-2023 (2007).

29. G. Niu, K.L. Wright, M. Huang, *et al.*, Constitutive Stat3 activity up-regulates VEGF expression and tumor angiogenesis, *Oncogene*, **21**, 2000-2008 (2002).

30. D. Hanahan, R.A. Weinberg, Hallmarks of cancer: The next generation, *Cell*, **144**(5), 646-674 (2011).

31. S. Ménard, S.M. Pupa, M. Campiglio, E. Tagliabue, Biologic and therapeutic role of HER2 in cancer, *Oncogene*, **22**(42), 6570-6578 (2003).

32. D. Hanahan, R.A. Weinberg, The hallmarks of cancer, *Cell*, **100**(1), 57-70 (2000).

33. A. Sorkin, L.K. Goh, Endocytosis and intracellular trafficking of ErbBs, *Exp. Cell. Res.*, **315**(4), 683-696 (2009).

34. G. Levkowitz, H. Waterman, E. Zamir, *et al.*, c-Cbl/Sli-1 regulates endocytic sorting and ubiquitination o the epidermal growth factor receptor, *Genes Dev.*, **12**, 3663-3674 (1998).

35. S.J. Rogers, K.J. Harrington, P. Rhys-Evans, P. O-Charoenrat, S.A. Eccles, Biological significance of c-erbB family oncogenes in head and neck cancer, *Cancer Metastasis Rev.*, **24**(1), 47-69 (2005).

36. S.V. Sharma, D.W. Bell, J. Settleman, D.A. Haber, Epidermal growth factor receptor mutations in lung cancer, *Nat. Rev. Cancer*, **7**(3), 169-181 (2007).

37. M. Mrhalova, J. Plzak, J. Betka, R. Kodet, Epidermal growth factor receptor - its expression and copy numbers of EGFR gene in patients with head and neck squamous cell carcinomas, *Neoplasma*, **52**(4), 338-343 (2005).

38. B. Kumar, K.G. Cordell, J.S. Lee, *et al.*, Response to therapy and outcomes in oropharyngeal cancer are associated with biomarkers including human papillomavirus, epidermal growth factor receptor, gender, and smoking, *Int. J. Radiat. Oncol. Biol. Phys.*, **69**(2 Suppl.), 109-111 (2007).

39. J.R. Grandis, D.J. Tweardy, Elevated levels of transforming growth factor alpha and epidermal growth factor receptor messenger RNA are early markers of carcinogenesis in head and neck cancer, *Cancer Res.*, **53**, 3579-3584 (1993).

40. M. Sibilia, R. Kroismayr, B.M. Lichtenberger, A. Natarajan, M. Hecking, M. Holcmann, The epidermal growth factor receptor: from development to tumorigenesis, *Differentiation*, **75**(9), 770-787 (2007).

41. R. Terragni, A.C. Gardini, S. Sabattini, *et al.*, EGFR, HER-2 and KRAS in canine gastric epithelial tumors: A potential human model? *PLoS One*, **9**(1), e85388 (2014).

42. R.J. Higgins, P.J. Dickinson, R.A. Lecouteur, *et al.*, Spontaneous canine gliomas: Overexpression of EGFR, PDGFRα and IGFBP2 demonstrated by tissue microarray immunophenotyping, *J. Neurooncol.*, **98**(1), 49-55 (2010).

43. R. Thomas, S.E. Duke, H.J. Wang, *et al.*, "Putting our heads together": Insights into genomic conservation between human and canine intracranial tumors, *J. Neurooncol.*, **94**(3), 333-349 (2009).

44. H.K. Gan, A.H. Kaye, R.B. Luwor, The EGFRvIII variant in glioblastoma multiforme, *J. Clin. Neurosci.*, **16**(6), 748-754 (2009).

45. J. Baselga, Why the epidermal growth factor receptor? The rationale for cancer therapy, *Oncologist*, **7**(Suppl. 4), 2-8 (2002).

46. L. Milas, K.A. Mason, K.K. Ang, Epidermal growth factor receptor and its inhibition in radiotherapy: *In vivo* findings, *Int. J. Radiat. Biol.*, **79**(7), 539-545 (2003).

47. M. Yan, M. Schwaederle, D. Arguello, HER2 expression status in diverse cancers : review of results, *Cancer Metastasis Rev.*, **34**(1), 157-164 (2015).

48. M.D. Williams, D.B. Roberts, M.S. Kies, L. Mao, R.S. Weber, A.K. El-Naggar, Genetic and expression analysis of HER-2 and EGFR genes in salivary duct carcinoma: Empirical and therapeutic significance, *Clin. Cancer Res.*, **16**(8), 2266-2274 (2010).

49. M. Yan, B.A. Parker, R. Schwab, R. Kurzrock, HER2 aberrations in cancer: Implications for therapy, *Cancer Treat. Rev.*, **40**(6), 770-780 (2014).

50. F. Cappuzzo, L. Bemis, M. Varella-Garcia, HER2 mutation and response to trastuzumab therapy in non-small-cell lung cancer, *N. Engl. J. Med.*, **354**(24), 2619-2621 (2006).

51. H. Zhou, R. Randall, A. Brothman, T. Maxwell, C. Coffin, R. Goldsby, Expression in osteosarcoma increases risk of lung metastasis and can be associated with gene amplification, *J. Pediatr. Hematol. Oncol.*, **25**(1), 27-32 (2003).

52. N.J. Mason, J.S. Gnanandarajah, J.B. Engiles, *et al.*, Immunotherapy with a HER2-targeting listeria induces HER2-specific immunity and demonstrates potential therapeutic effects in a phase I trial in canine osteosarcoma**,** *Clin Cancer Res.*, **22**(17), 4380-4390 (2016).

53. C.A. London, Tyrosine kinase inhibitors in veterinary medicine, *Top. Companion Anim. Med.*, **24**(3), 106-112 (2009).

54. R. Klopfleisch, P. Klose, A.D. Gruber, The combined expression pattern of BMP2, LTBP4, and DERL1 discriminates malignant from benign canine mammary tumors, *Vet. Pathol.*, **47**(3), 446-454 (2010).

55. J.S. Looper, D.E. Malarkey, D. Ruslander, D. Proulx, D.E. Thrall, Epidermal growth factor receptor expression in feline oral squamous cell carcinomas, *Vet. Comp. Oncol.*, **4**(1), 33-40 (2006).

56. Z. Grabarević, M. Corić, S. Seiwerth, *et al.*, Comparative analysis of hepatocellular carcinoma in men and dogs, *Coll. Antropol.*, **33**(3), 811-814 (2009).

57. G. Stoica, H.-T. Kim, D.G. Hall, J.R. Coates, Morphology, immunohistochemistry, and genetic alterations in dog astrocytomas, *Vet. Pathol.*, **41**(1), 10-19 (2004).

58. D.L. Ipsitz, R.J.H. Iggins, G.D.K. Ortz, *et al.*, Glioblastoma multiforme: clinical findings, magnetic resonance imaging , and pathology in five dogs, *Vet. Pathol.*, **40**(6), 659-669 (2003).

59. A.A. Santos, A.J.F. Matos, Advances in the understanding of the clinically relevant genetic pathways and molecular aspects of canine mammary tumours. Part 2: Invasion, angiogenesis, metastasis and therapy, *Vet. J.*, **205**(2), 144-153 (2015).

60. M.I. Carvalho, M.J. Guimarães, I. Pires, *et al.*, EGFR and microvessel density in canine malignant mammary tumours, *Res. Vet. Sci.*, **95**(3), 1094-1099 (2013).

61. A. Gama, F. Gärtner, A. Alves, F. Schmitt, Immunohistochemical expression of Epidermal Growth Factor Receptor (EGFR) in canine mammary tissues, *Res. Vet. Sci.*, **87**(3), 432-437 (2009).

62. N.H. Kim, H.Y. Lim, K.S. Im, J.H. Kim, J.-H. Sur, Identification of triple-negative and basal-like canine mammary carcinomas using four basal markers, *J. Comp. Pathol.*, **148**(4), 298-306 (2013).

63. F. Sassi, C. Benazzi, G. Castellani, G. Sarli, Molecular-based tumour subtypes of canine mammary carcinomas assessed by immunohistochemistry, *BMC Vet. Res.*, **6**, 5 (2010).

64. A. Gama, A. Alves, F. Schmitt, Identification of molecular phenotypes in canine mammary carcinomas with clinical implications: Application of the human classification, *Virchows Arch.*, **453**(2), 123-132 (2008).

65. F.L. Queiroga, M.D. Perez-Alenza, A. González-Gil, G. Silván, L. Peña, J.C. Illera, Quantification of epidermal growth factor receptor (EGFR) in canine mammary tumours by ELISA assay: Clinical and prognostic implications, *Vet. Comp. Oncol.*, **15**(2), 383-390 (2017).

66. J. Martín de las Mulas, J. Ordás, Y. Millán, V. Fernández-Soria, S. Ramón y Cajal, Oncogene HER-2 in canine mammary gland carcinomas: An immunohistochemical and chromogenic in situ hybridization study, *Breast Cancer Res. Treat.*, **80**, 363-367 (2003).

67. F. Millanta, J. Impellizeri, L. McSherry, G. Rocchigiani, L. Aurisicchio, G. Lubas, Overexpression of HER-2 via immunohistochemistry in canine urinary bladder transitional cell carcinoma - A marker of malignancy and possible therapeutic target, *Vet. Comp. Oncol.*, **16**(2), 297-300 (2018).

68. A.F. Flint, L. U'Ren, M.E. Legare, S.J. Withrow, W. Dernell, W.H. Hanneman, Overexpression of the erbB-2 proto-oncogene in canine osteosarcoma cell lines and tumors, *Vet. Pathol.*, **41**(3), 291-296 (2004).

69. H. Murua-Escobar, K. Becker, J. Bullerdiek, I. Nolte, The canine ERBB2 gene maps to a chromosome region frequently affected by aberrations in tumors of the dog (*Canis familiaris*), *Cytogenet. Cell Genet.*, **94**(3-4), 194-195 (2001).

70. P. Wu, T.E. Nielsen, M.H. Clausen, FDA-approved small-molecule kinase inhibitors, *Trends Pharmacol. Sci.*, **36**(7), 422-439 (2015).

71. R.A. Norman, D. Toader, A.D. Ferguson, Structural approaches to obtain kinase selectivity, *Trends Pharmacol. Sci.*, **33**(5), 273-278 (2012).

72. M.E.M. Noble, J.A. Endicott, L.N. Johnson, Protein kinase inhibitors: insights into drug design from structure, *Science*, **303**(5665), 1800-1805 (2004).

73. A.J. Tevaarwerk, J.M. Kolesar, Lapatinib: A small-molecule inhibitor of epidermal growth factor receptor and human epidermal growth factor receptor-2 tyrosine kinases used in the treatment of breast cancer, *Clin. Therapeut.*, **31**(Part. 2), 2332-2348 (2009).

74. E.R. Wood, A.T. Truesdale, O.B. McDonald, *et al.*, A unique structure for epidermal growth factor receptor bound to GW572016 (Lapatinib): Relationships among protein conformation, inhibitor off-rate, and receptor activity in tumor cells, *Cancer Res.*, **64**(18), 6652-6659 (2004).

75. C. Qiu, M.K. Tarrant, S.H. Choi, *et al.*, Mechanism of activation and inhibition of the HER4/ErbB4 kinase, *Structure*, **16**(3), 460-467 (2008).

76. H.-R. Tsou, E.G. Overbeek-klumpers, W.A. Hallett, *et al.*, Optimization of 6,7-disubstituted-4-(arylamino)quinoline-3-carbonitriles as orally active, irreversible inhibitors of human epidermal growth factor receptor-2 kinase activity, *J. Med. Chem.*, **48**(4), 1107-1131 (2005).

77. S.K. Rabindran, C.M. Discafani, E.C. Rosfjord, *et al.*, Antitumor activity of HKI-272, an orally active, irreversible inhibitor of the HER-2 tyrosine kinase, *Cancer Res.*, **64**(11), 3958-3965 (2004).

78. A. Wissner, T.S. Mansour, The vevelopment of HKI-272 and related compounds for the treatment of cancer, *Arch. Pharm. Chem. Life Sci.*, **341**(8), 465-477 (2008).

79. S.R. Tiwari, P. Mishra, J. Abraham, Neratinib, a novel HER2-targeted tyrosine inhibitor, *Clin. Breast Cancer*, **16**(5), 344-348 (2016).

80. F. Solca, G. Dahl, A. Zoephel, *et al.*, Target binding properties and cellular activity of afatinib (BIBW 2992), an irreversible ErbB family blocker, *J. Pharmacol. Exp. Ther.,* **343**(2), 342-350 (2012).

81. R. Roskoski, Jr., ErbB/HER protein-tyrosine kinases: Structures and small molecule inhibitors, *Pharmacol. Res.*, **87**, 42-59 (2014).

82. S.A. Hurvitz, R. Shatsky, N. Harbeck, Afatinib in the treatment of breast cancer, *Expert Opin. Investig. Drugs*, **23**(7), 1039-1047 (2014).

83. D. Li, L. Ambrogio, T. Shimamura, *et al.*, BIBW2992, an irreversible EGFR/HER2 inhibitor highly effective in preclinical lung cancer models, Oncogene, 27(34), 4702-4711 (2008).

84. Y. Wu, Y. Zhang, M. Wang, *et al.*, Downregulation of HER3 by a novel antisense oligonucleotide, EZN-3920, improves the antitumor activity of EGFR and HER2 tyrosine kinase inhibitors in animal models, *Mol. Cancer Ther.*, **12**(4), 427-437 (2013).

85. A. Canonici, K. Pedersen, B. Browne, M. McDermott, N. Walsh, J. Crown, N. O'Donovan, Effect of afatinib alone and in combination with trastuzumab in HER2-positive breast cancer cell lines, *J. Clin. Oncol.*, **31**(15 Suppl.), 632 (2013).

86. R.L.N. Godone, G.M. Leitão, N.B. Araújo, C.H.M. Castelletti, J.L. Lima-Filho, D.B.G. Martins, Clinical and molecular aspects of breast cancer : Targets and therapies, *Biomed. Pharmacother.*, **106**, 14-34 (2018).

87. X. Zhang, P.N. Munster, New protein kinase inhibitors in breast cancer: afatinib and neratinib, *Expert Opin. Pharmacother.*, **15**(9), 1277-1288 (2014).

88. J.A. Engelman, K. Zejnullahu, C. Gale, *et al.*, PF00299804, an irreversible Pan-ERBB inhibitor, is effective in lung cancer models with EGFR and ERBB2 mutations that are resistant to gefitinib, *Cancer Res.*, **67**(24), 11924-11933 (2007).

89. A.J. Gonzales, K.E. Hook, I.W. Althaus, *et al.*, Antitumor activity and pharmacokinetic properties of PF-00299804 , a second-generation irreversible pan-erbB receptor tyrosine kinase inhibitor, *Mol. Cancer Ther.*, **7**(7), 1880-1889 (2008).

90. B.-C. Liao, C.-C. Lin, J.C.-H. Yang, Second and third-generation epidermal growth factor receptor tyrosine kinase inhibitors in advanced nonsmall cell lung cancer, *Curr. Opin. Oncol.*, **27**(2), 94-101 (2015).

91. S.S. Ramalingam, F. Blackhall, M. Krzakowski, *et al.*, Randomized phase II study of dacomitinib (PF-00299804), an irreversible pan-human epidermal growth factor receptor inhibitor, versus erlotinib in patients with advanced non-small-cell lung cancer, *J. Clin. Oncol.*, **30**(27), 3337-3344 (2012).

92. J. Singer, M. Weichselbaumer, T. Stockner, *et al.*, Comparative oncology: ErbB-1 and ErbB-2 homologues in canine cancer are susceptible to cetuximab and trastuzumab targeting, *Mol. Immunol.*, **50**(4), 200-209 (2012).

93. K. Lindblad-Toh, C.M. Wade, T.S. Mikkelsen, *et al.*, Genome sequence, comparative analysis and haplotype structure of the domestic dog, *Nature*, **438**(7069), 803-819 (2005).

94. M.P. Hoeppner, A. Lundquist, M. Pirun, et al., An improved canine genome and a comprehensive catalogue of coding genes and non-coding transcripts, *PLoS One*, **9**(3), e91172 (2014).

95. P. Rivera, H. von Euler, Molecular biological aspects on canine and human mammary tumors, *Vet. Pathol.*, **48**(1), 132-146 (2011).

96. S. Visan, O. Balacescu, I. Berindan-Neagoe, C. Catoi, *In vitro* comparative models for canine and human breast cancer, *Clujul Med.*, **89**(1), 38-49 (2016).

97. K.S. Borge, S. Nord, P. Van-Loo, *et al.*, Canine mammary tumours are affected by frequent copy number aberrations, Including amplification of MYC and loss of PTEN, *PLoS One*, **10**(5), e0126371 (2015).

98. F.L. Queiroga, T. Raposo, M.I. Carvalho, J. Prada, I. Pires, Canine mammary tumours as a model to study human breast cancer: most recent findings, *In Vivo*, **25**(3), 455-465 (2011).

99. C.A. London, A.L. Hannah, R. Zadovoskaya, *et al.*, Phase I dose-escalating study of SU11654, a small molecule receptor tyrosine kinase inhibitor, in dogs with spontaneous malignancies, *Clin. Cancer Res.*, **9**(7), 2755-2768 (2003).

100. G.S. Papaetis, K.N. Syrigos, Sunitinib: A multitargeted receptor tyrosine kinase inhibitor in the era of molecular cancer therapies, *BioDrugs*, **23**(6), 377-389 (2009).

101. C.A. London, Small molecule inhibitors in veterinary oncology practice, *Vet. Clin. North Am., Small Anim. Pract.*, **44**(5), 893-908 (2014).

102. C. London, T. Mathie, N. Stingle, *et al.*, Preliminary evidence for biologic activity of toceranib phosphate (Palladia®) in solid tumours, *Vet. Comp. Oncol.*, **10**(3), 194-205 (2012).

103. A. Marcinowska, J. Warland, M. Brearley, J. Dobson, A novel approach to treatment of lymphangiosarcoma in a boxer dog, *J. Small Anim. Pract.*, **54**(6), 334-337 (2013).

104. E. Chon, L. McCartan, L.N. Kubicek, D.M. Vail, Safety evaluation of combination toceranib phosphate (Palladia) and piroxicam in tumour-bearing dogs (excluding mast cell tumours): A phase I dose-finding study, *Vet. Comp. Oncol.*, **10**(3), 184-193 (2012).

105. C. Robat, C. London, L. Bunting, *et al.*, Safety evaluation of combination vinblastine and toceranib phosphate (Palladia®) in dogs: A phase I dose-finding study, *Vet. Comp. Oncol.*, **10**(3), 174-183 (2012).

106. K.S. Carlsten, C.A. London, S. Haney, R. Burnett, A.C. Avery, D.H. Thamm, Multicenter prospective trial of hypofractionated radiation treatment, toceranib, and prednisone for measurable canine mast cell tumors, *J. Vet. Intern. Med.*, **26**(1), 135-141 (2012).

107. L. Marconato, G. Bettini, C. Giacoboni, *et al*., Clinicopathological features and outcome for dogs with mast cell tumors and bone marrow involvement, *J. Vet. Intern. Med.*, **22**(4), 1001-1007 (2008).

108. M. Isotani, N. Ishida, M. Tominaga, *et al.*, Effect of tyrosine kinase inhibition by imatinib mesylate on mast cell tumors in dogs, *J. Vet. Intern. Med.*, **22**(4), 985-988 (2008).

109. O. Yamada, M. Kobayashi, O. Sugisaki, *et al.*, Imatinib elicited a favorable response in a dog with a mast cell tumor carrying a c-kit c.1523A>T mutation via suppression of constitutive KIT activation, *Vet. Immunol. Immunopathol.*, **142**(1-2), 101-106 (2011).

How to cite this article

J.M. Cadena-García, C.E. Giraldo-Murillo, M. Ramos Jaramillo, EGFR and HER2 small molecules inhibitors as potential therapeutics in veterinary oncology, *Rev. Colomb. Cienc. Quím. Farm.*, **49**(2), 452-471 (2020).