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Carcinogenicity risk assessment of romosozumab: A review of scientific weight-of-evidence and findings in a rat lifetime pharmacology study

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ABSTRACT

Romosozumab is a humanized immunoglobulin G_2 monoclonal antibody that binds and blocks the action of sclerostin, a protein secreted by the osteocyte and an extracellular inhibitor of canonical Wnt signaling. Blockade of sclerostin binding to low-density lipoprotein receptor-related proteins 5 and 6 (LRP5 and LRP6) allows Wnt ligands to activate canonical Wnt signaling in bone, increasing bone formation and decreasing bone resorption, making sclerostin an attractive target for osteoporosis therapy. Because romosozumab is a bone-forming agent and an activator of canonical Wnt signaling, questions have arisen regarding a potential carcinogenic risk. Weight-of-evidence factors used in the assessment of human carcinogenic risk of romosozumab included features of canonical Wnt signaling, expression pattern of sclerostin, phenotype of loss-of-function mutations in humans and mice, mode and mechanism of action of romosozumab, and findings from romosozumab chronic toxicity studies in rats and monkeys. Although the weight-of-evidence factors supported that romosozumab would pose a low carcinogenic risk to humans, the carcinogenic potential of romosozumab was assessed in a rat lifetime study. There were no romosozumab-related effects on tumor incidence in rats. The findings of the lifetime study and the weight-of-evidence factors collectively indicate that romosozumab administration would not pose a carcinogenic risk to humans.

1. Introduction

Romosozumab is a humanized immunoglobulin (Ig) G_2 monoclonal antibody (Ab) that binds and blocks the action of sclerostin, a protein secreted by osteocytes that is an extracellular inhibitor of canonical Wnt signaling and bone formation (Poole et al., 2005; van Bezooijen et al., 2004). Using Kinetic Exclusion Assay technology, romosozumab has been found to bind to human, cynomolgus monkey, and rat sclerostin with measured dissociation constants (K_d) of 11 pM, 23 pM, and 3 pM, respectively (Gong et al., 2016). Blockade of sclerostin binding by romosozumab to low-density lipoprotein receptor-related proteins 5 and 6 (LRP5 and LRP6) allows Wnt ligands to activate canonical Wnt signaling in bone and increase bone formation (Baron and Kneissel, 2013; Gong et al., 2016). Activation of canonical Wnt signaling by romosozumab or a rat surrogate Ab with the same complementarity-determining region (sclerostin Ab [Scl-Ab]) has been demonstrated in vitro and in vivo (Gong et al., 2016; Nioi et al., 2015; Taylor et al., 2016). Currently under investigation in clinical trials, romosozumab administered at 210 mg once monthly for 12 months has been demonstrated to increase bone formation markers and bone mass in humans (McClung et al., 2014).

Consistent with the high bone mass phenotype of homozygous and heterozygous mouse models and human loss-of-function (LOF) *Sclerostin* (*SOST*) mutations, romosozumab or Scl-Ab increases bone formation, mass, and strength in ovariectomized rats, aged male rats, and gonad-intact female and male cynomolgus monkeys (Li

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Abbreviations

Ab	antibody
aBMD	areal bone mineral density
aBMC	areal bone mineral content
ADA	antidrug antibody
ANOVA	analysis of variance
AUC	area under the curve
BFR/BS	bone formation rate, surface referent
BL	baseline
BMC	bone mineral content
BMD	bone mineral density
CA	serum calcium
DJD	degenerative joint disease
DXA	dual-energy X-ray absorptiometry
Ec	endocortical
FDA	Food and Drug Administration
GLU	glucose
GSK3	glycogen synthase kinase 3
HBM	high bone mass
hPTH	human parathyroid hormone
HT	hematocrit
ICH	International Council for Harmonisation
Ig	immunoglobulin
IHC	immunohistochemistry
ISH	in situ hybridization
L	labeled
LOF	loss-of-function
LRP	low-density lipoprotein receptor-related protein
MPV	mean platelet volume
MS/BS	mineralizing surface/bone surface
NOAEL	no-observed-adverse-effect-level
OSA	osteosarcoma
PHOS	phosphorus
PLT	platelets
Pm	perimeter
pQCT	peripheral quantitative computed tomography
Ps	periosteal
PTH	parathyroid hormone
QM	once monthly
QW	once a week
RBC	red blood cells
RETIC	reticulocytes
RNA-Seq	ribonucleic acid sequencing
RT-PCR	reverse transcriptase polymerase chain reaction
SC	subcutaneous
Scl-Ab	Sclerostin antibody
SD	Sprague-Dawley
SD	standard deviation
SEM	standard error of the mean
SOST	Sclerostin
vBMC	volumetric bone mineral content
wk	week
yr	year

et al., 2014, 2009, 2010; Ominsky et al., 2011; Ominsky et al., 2010). In animal models, sclerostin inhibition by a Scl-Ab results in a decrease or no change in bone resorption (Li et al., 2010; Ominsky et al., 2011; Stolina et al., 2014). In humans, increases in bone mass are associated with transient increases in bone formation markers and sustained decreases in resorption markers (McClung et al., 2014; Padhi et al., 2011).

Because romosozumab is a bone-forming agent and an activator of canonical Wnt signaling, questions have arisen regarding a potential carcinogenic risk (Baron and Hesse, 2012; Hoeppner et al., 2009; Lewiecki, 2014; Schett and Bozec, 2014). These concerns stem from both the association of mutations in the canonical Wnt pathways with human cancers arising through activating mutations or epigenetic changes in the intracellular signaling components (Anastas and Moon, 2013; Kansara et al., 2009; Polakis, 2007), and experimental overexpression of Wnt ligands, which results in hyperplasia followed by neoplasia (Bradbury et al., 1995; Lane and Leder, 1997; Nusse and Varmus, 1982; Roelink et al., 1990). However, inactivating mutations in genes thought to inhibit Wnt signaling (notably extracellular inhibitors) have generally not been associated with cancer but rather with non-neoplastic disorders (Anastas and Moon, 2013). Bone neoplastic findings have been observed with other bone-forming agents, specifically human parathyroid hormone (hPTH; hPTH [1–84] and other analogues). In 2-year rat studies with hPTH and other analogues and a hPTH-related protein analogue, a high incidence of osteosarcomas (OSAs) associated with preneoplastic bone changes (osteoblast hyperplasia and stromal proliferation) and benign bone tumors was observed in both F344 and Sprague-Dawley (SD) rats, with incidence increasing with dose (Jolette et al., 2006, 2014; Vahle et al., 2002; Watanabe et al., 2012). For this reason, the prescribing information for the only Food and Drug Administration (FDA)–approved bone-forming agent teriparatide (hPTH [1–34]) has a black box warning for potential risk of OSA.

Many factors contribute to the overall assessment of the human carcinogenic risk of romosozumab based on a weight-of-evidence approach described by an International Council for Harmonisation (ICH) S1 Expert Working Group for small molecule drugs—ICH S1 regulatory notice 10 March 2015 (ICH, 2015). Key factors contributing to the weight-of-evidence for carcinogenic potential for romosozumab include features of canonical Wnt signaling, the restricted expression pattern of sclerostin, human and mouse genetic LOF data, the mode and molecular mechanism of action of romosozumab compared with other bone-forming agents, and findings in the chronic toxicology studies in rats and cynomolgus monkeys. Key data for each of these factors are provided below, with more detailed information provided in the Supplemental Materials section 2:

- As a morphogen pathway during development, canonical Wnt signaling evolved to be a highly spatially and temporally regulated autocrine/paracrine signaling pathway (Supplemental Materials section 2.1).
- As an autocrine/paracrine pathway, activation of canonical Wnt signaling will be restricted to tissues that express sclerostin. The tissue expression pattern of sclerostin is limited largely to cells encased in a mineralized matrix, with the major site of expression in osteocytes (Table S1).
- The bone-specific phenotype of LOF *SOST* mutations in humans and mice further supports the largely restricted effects of loss or deficiency of sclerostin to bone, with no reported increases in cancer in either humans or mice with LOF mutations (Supplemental Materials section 2.2).
- Romosozumab has a unique mode of action at the bone tissue level compared with the only approved bone-forming agent teriparatide (hPTH [1–34]). In contrast to teriparatide, which