NTRK Gene Fusions and TRK Inhibitors BluePrint

This BluePrint has been developed under the guidance of a steering committee which included the following members:

- *Prof. Andrea Sartore-Bianchi a medical oncologist at Ospedale Niguarda and University of Milano, Italy*
- *Prof. Fernando Lopez-Rios a molecular and surgical pathologist at Hospital Universitario HM Sanchinarro, Spain*
- *Assoc. Prof. Gerald Prager a medical oncologist at the Medical University of Vienna, Austria*
- *Dr. Jaclyn Hechtman a molecular and surgical pathologist at Memorial Sloan Kettering Cancer Center, USA*

KEY CLINICAL POINTS

- *NTRK* gene fusions are targetable genetic alterations that code for targetable fusion proteins and drive tumourigenesis
- *NTRK* gene fusions can occur either at a high frequency in some rare cancers (high-prevalence tumours) or at a low frequency in more common cancer types (low-prevalence tumours)
- Tumour-agnostic treatment is a genetically informed treatment strategy that enriches for novel targets regardless of tumour histology
- TRK inhibitors are a form of tumour-agnostic treatment
  - Two TRK inhibitors are currently approved for use in clinical practice (larotrectinib and entrectinib)
  - Other TRK inhibitors are in clinical development
- A testing algorithm for *NTRK* gene fusions has been proposed to identify patients who would benefit from therapies targeting TRK fusion proteins:
  - FISH or RT-PCR are recommended for patients with a tumour type associated with a high prevalence of *NTRK* gene fusions
  - For low-prevalence tumours, NGS is recommended
  - If NGS is not available, IHC can be used as a screening tool
- Once a patient has been identified as having a *NTRK* gene fusion, treatment with an approved TRK inhibitor is recommended

Document Purpose

To provide a brief introduction and reference guide to key aspects of the importance of TRK fusion proteins as a therapeutic target and its therapeutic potential in cancer, including recommendations on testing for *NTRK* gene fusions. More information can be found on the *NTRK* gene fusions ESMO OncologyPRO portal that has been developed in parallel with this BluePrint.

Disclaimer

This content is supported by an Independent Educational Grant from Bayer and is and under the sole responsibility of the experts in the steering committee.

NTRK family

TRK proteins are a family of three transmembrane receptor tyrosine kinases (TRKA, TRKB, and TRKC) encoded by the *NTRK1*, *NTRK2*, and *NTRK3* genes, respectively [1]. The binding of the ligand (as homodimers) to the corresponding TRK receptor induces the dimerization of the receptor and autophosphorylation of the tyrosine kinase (TK) domain (see figure 1).

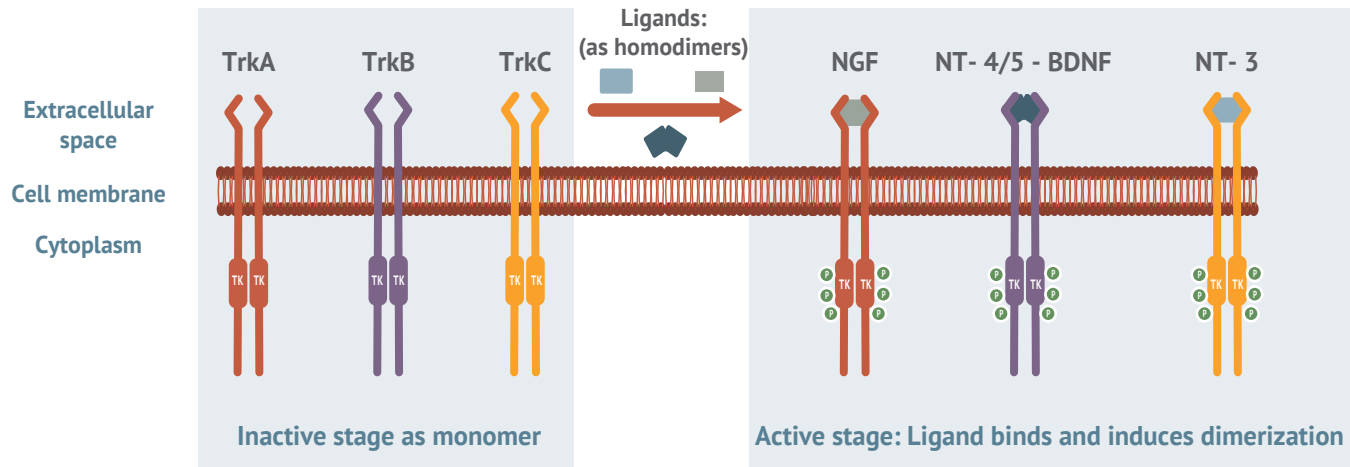

Figure 1. Activation of TRK receptors by binding to their ligands. *NGF (nerve growth factor) binds to TRKA, NT-4/5 (neurotrophin 4/5) and BDNF (brain-derived neurotrophic factor) to TRKB and NT-3 (neurotrophin-3) binds to TRKC inducing the dimerization of the receptors and autophosphorylation (represented by "P") of their tyrosine kinase domain (TK).*

The dimerization of the receptors and phosphorylation of their TK domain induce the activation of three signalling pathways (PKC, PI3K and MAPK pathways) involved in proliferation, differentiation and survival (see figure 2).

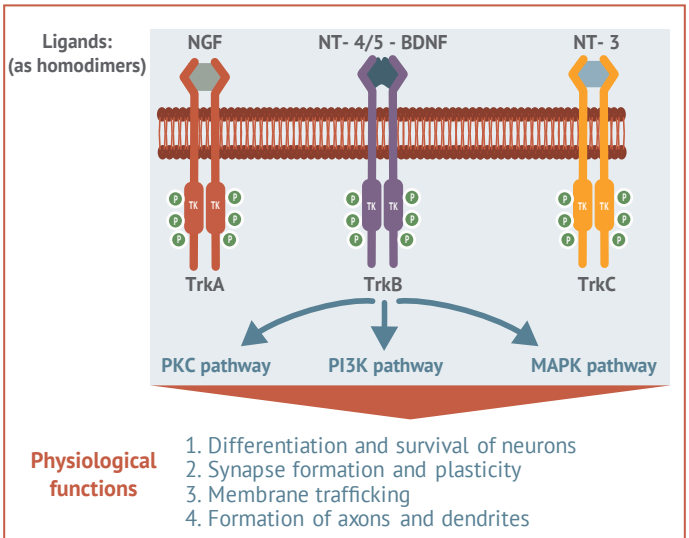

Figure 2. Signalling activation through TRK family. *Activation of TRK receptors induces activation of three signalling pathways (PKC, PI3K and MAPK) resulting in the physiological functions of the TRK protein receptor family.*

NTRK Gene Fusions Are Targetable Genetic Alterations That Drive Tumourigenesis

NTRK gene fusions are targetable genetic alterations that code for fusion proteins and drive tumourigenesis [1-3].

All identified *NTRK* gene fusions involve joining of the 3' region of the *NTRK* gene, including the tyrosine-kinase domain, with the 5' region of a different gene (i.e. the fusion partner) by intra- or inter-chromosomal rearrangement (see figures 3 and 4) [1, 4]. The protein product of *NTRK* gene fusions (referred to as a TRK fusion protein) is constitutively active and results in cell growth, proliferation, and survival pathway activation (see figure 3) [1, 4, 5].

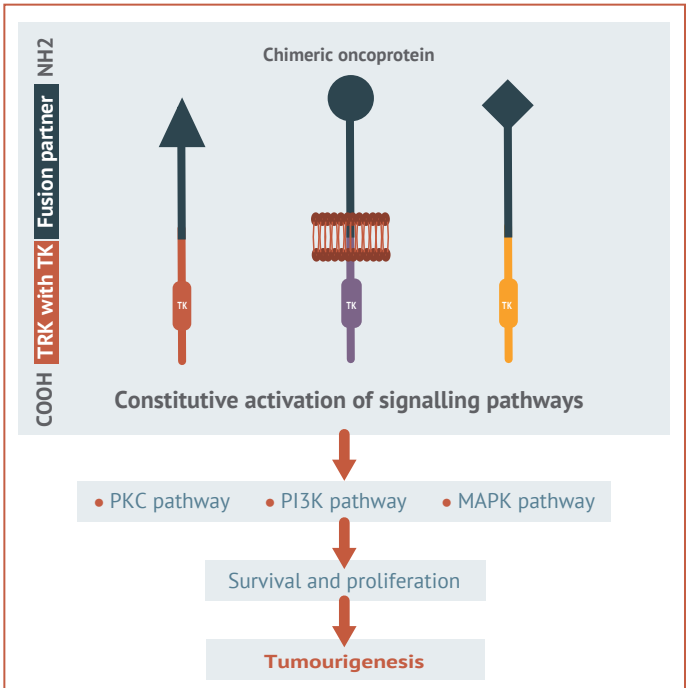

Figure 3. NTRK gene fusions characteristics and induction of tumourigenesis. *TRK fusion protein is constitutively activated resulting in tumourigenesis functions through permanent activation of three signalling pathways (PKC, PI3K and MAPK).*

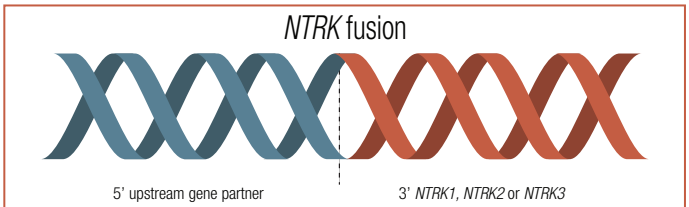

Figure 4. NTRK gene fusions structure. *Fusion of the 3' region of the NTRK gene with the 5' region of a gene partner.*

Frequency And Sites of *NTRK* Gene Fusions in Adult and Paediatric Cancers [1][a]

The estimated frequency of *NTRK* gene fusions varies between tumour types ranging from a prevalence of >90% in some rare cancers (breast secretory carcinoma and infantile fibrosarcoma – known as high-prevalence tumours) to <5% in more common cancer types – known as low-prevalence tumours (see figure 5) [1, 2, 4].

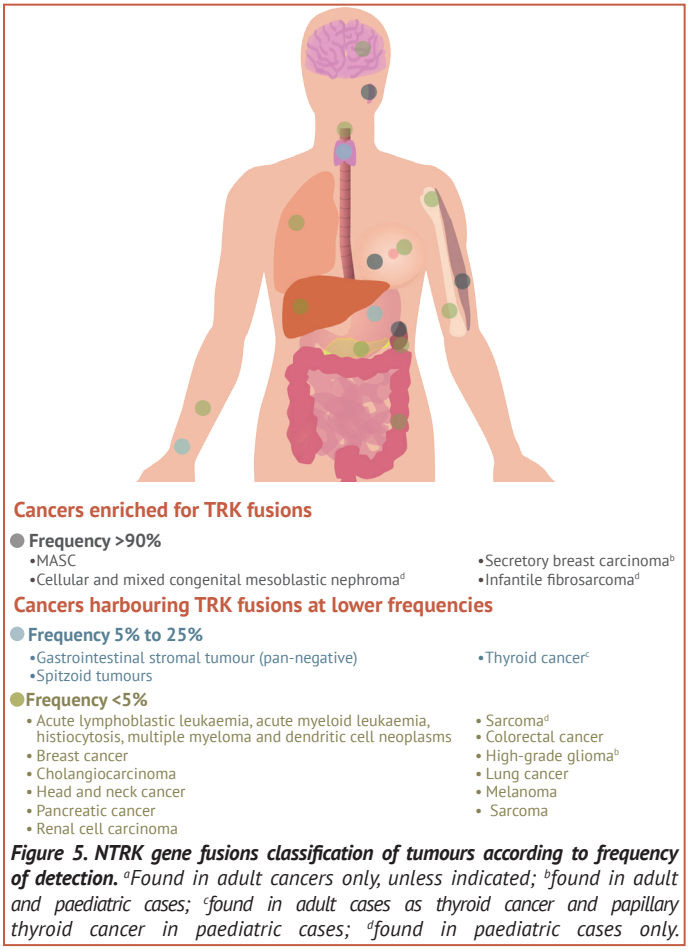

Figure 5. NTRK gene fusions classification of tumours according to frequency of detection. *[a]Found in adult cancers only, unless indicated; [b]found in adult and paediatric cases; [c]found in adult cases as thyroid cancer and papillary thyroid cancer in paediatric cases; [d]found in paediatric cases only.*

Testing for *NTRK* gene fusions

- Testing for gene fusions allows for the identification of patients who may benefit from gene-specific inhibitor therapy
- Target enrichment strategies: allows for the rapid screening of a large patient population to identify a small percentage who have gene fusions

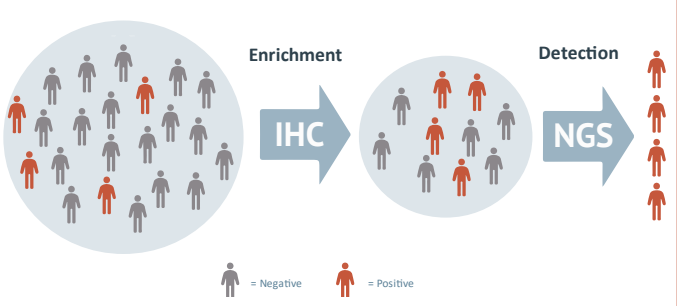

Figure 6. Gene fusions: enrichment strategies and detection methods

Techniques used to detect *NTRK* gene fusions at DNA and RNA levels include DNA-based next-generation sequencing (NGS), RNA-based NGS, reverse-transcriptase PCR (RT-PCR), fluorescence *in situ* hybridisation (FISH), and at protein level include immunohistochemistry (IHC).

- Several challenges exist when testing for TRK fusion cancer and each test methodology has advantages and disadvantages in terms of resource efficiency, turnaround time, sensitivity, and specificity which must be balanced with the individual case in mind.
- Details on each specific technique are provided in Module 2 of the *NTRK* gene fusion ESMO OncologyPRO portal
- A general algorithm for *NTRK* gene fusions testing based on recent guidelines to identify patients who would benefit from therapies targeting TRK fusion proteins is outlined (see figure 7).
  - FISH or RT-PCR are recommended for patients with a histologic tumour type associated with high-prevalence *NTRK* gene fusions.
  - For low-prevalence tumours, NGS is recommended.
  - If NGS is not available, IHC can be used as a screening tool.

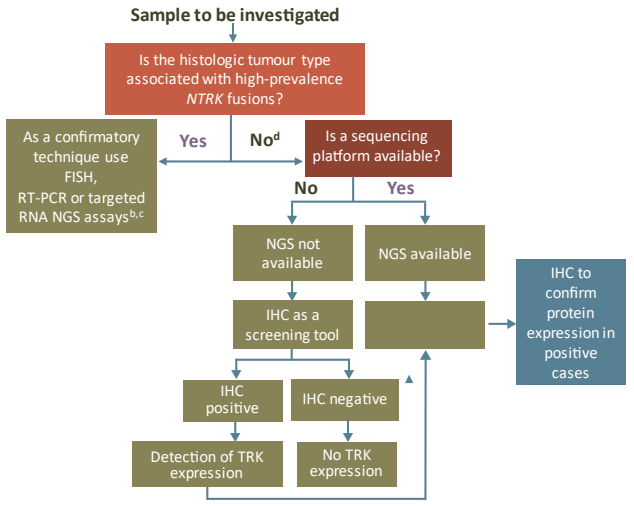

Figure 7. Recommendations of NTRK gene fusions testing algorithm. *[a]Based on ESMO 2019 guidelines for NTRK gene fusion detection and guidelines for TRK fusion cancer in children by Albert et al. 2019; [b]Using specific probes for the rearrangement involving the known NTRK gene; [c]Albert et al., note that RT-PCR is not routinely used in clinical practice and limited data are available using this technique for NTRK gene fusions detection; [d]ESMO guidelines note that this population would be likely represented by any malignancy at an advanced stage, in particular if it has been proven wild type for other known genetic alterations tested in routine practice, and especially if diagnosed in young patients. (FISH, fluorescence in situ hybridization; IHC, immunohistochemistry; RT-PCR, reverse transcriptase-polymerase chain reaction.) Algorithm for NTRK gene fusions testing [6-8][a]*

Tumour-Agnostic Treatments

Tumour-agnostic treatment is a genetically informed treatment strategy that enriches for novel targets regardless of tumour site of origin. Such treatments are based on the observation that the same molecular alteration may be present in several cancer types and that molecular or genetic alterations essential for the growth of cancer of one tissue origin (based on preclinical studies in cell culture and animal models) have been found to be important for another.

A therapy successful against a target in one cancer type may therefore prove effective for the same target in a different cancer type. [9]

Two TRK inhibitors are currently approved for use in clinical practice (larotrectinib and entrectinib); Other TRK inhibitors are in clinical development

TRK inhibitors differ in their stage of development, characteristics, and mechanism of action, as well as whether they address acquired resistance to TRK protein inhibition. Key points include:

- First-generation TRK inhibitors include larotrectinib and entrectinib:
  - Larotrectinib was the first TRK-directed therapy to receive approval by US FDA in November 2018 under accelerated approval based on the innovative concept of a tumour-agnostic indication via a priority pathway [10][11]. Larotrectinib was approved for the treatment of adult and paediatric patients with solid tumours that have a *NTRK* gene fusion without a known acquired resistance mutation, are metastatic or where surgical resection is likely to result in severe morbidity, and have no satisfactory alternative treatments or that have progressed following treatment [12]. Following the US approval, ANVISA in Brazil issued an approval on 9 July 2019 [13] and Health Canada a notice of compliance with conditions on 16 July 2019 [14]. On 19 September 2019, larotrectinib received the EU conditional marketing authorization approval for the treatment of adult and paediatric patients with solid tumours that display a *NTRK* gene fusion [15].
  - In June 2019, entrectinib was approved in Japan for the treatment of adult and paediatric patients *NTRK* gene fusions-positive, advanced recurrent solid tumours [16]. In August 2019, entrectinib was approved for the treatment of adults and paediatric patients ⩾12 years of age with solid tumours that have a *NTRK* gene fusion without a known acquired resistance mutation, are metastatic or where surgical resection is likely to result in severe morbidity, and have progressed following treatment or have no satisfactory standard therapy [17].
- Next-generation TRK inhibitors include seletrectinib (formely known as LOXO-195) and repotrectinib.
- Other TRK inhibitors are under clinical investigation. These are either multikinase inhibitors with anti-TRK activity or drugs that are specifically TRK-directed and include cabozantinib, merestinib, sitravatinib, ONO-7579, PLX-7486, altiratinib and DS-6051b.
- Response rates of up to 75% have been observed with TRK inhibition in *NTRK*-fusion driven cancers [10].

Clinical efficacy data for larotrectinib and entrectinib are based on the US Prescribing information labels and are summarized below and in the table 1 [12 and 19]:

- **The efficacy and safety of larotrectinib** are based on paediatric and adult patients with unresectable or metastatic solid tumours with a *NTRK* gene fusion enrolled in one of three multicenter, open-label, single-arm clinical trials: Study LOXO-TRK-14001 (NCT02122913), SCOUT (NCT02637687), and NAVIGATE (NCT02576431). (Cut off date July 2017 for the NDA approval).
- **The efficacy and safety of entrectinib** are based on adult patients with unresectable or metastatic solid tumours with a *NTRK* gene fusion enrolled in one of three multicenter, single-arm, open-label clinical trials: ALKA, STARTRK-1 (NCT02097810) and STARTRK-2 (NCT02568267).

Table 1. Clinical efficacy data for the FDA-approved TRK inhibitors.

Efficacy Parameters	**Larotrectinib (N=55)[1]**	**Entrectinib (N=54)[1]**
Overall response rate (95% CI)	75% (61%, 85%)	57% (43, 71)
Complete response rate	22%	7.4%
Partial response rate	53%	50%
Duration of response[2]	**Larotrectinib (N=41)[1]**	**Entrectinib (N=31)[1]**
Range (months)	1.6+, 33.2+	2.8, 26.0+
% with duration ⩾ 6 months	73%	68%
% with duration ⩾ 9 months	63%	61%
% with duration ⩾ 12 months	39%	45%

[1]Disclaimer: Efficacy data presentation in the table come from the US FDA approved labels, differences in trial design do not allow for direct comparison of efficacy data (larotrectinib vs entrectinib)

[2]For larotrectinib: Median duration of response not reached at time of data cutoff

The clinical safety profile for larotrectinib and entrectinib are based on the US Prescribing information labels and are summarized below [12 and 18].

- **The safety profile of larotrectinib:**
  - The most common adverse reactions (≥ 20%) were fatigue, nausea, dizziness, vomiting, anaemia, increased AST, cough, increased ALT, constipation, and diarrhoea.
  - The most common serious adverse reactions (≥ 2%) were pyrexia, diarrhoea, sepsis, abdominal pain, dehydration, cellulitis, and vomiting.
  - Grade 3 or 4 adverse reactions occurred in 51% of patients.
- **The safety profile of entrectinib:**
  - The most common adverse reactions (≥ 20%) were fatigue, constipation, dysgeusia, oedema, dizziness, diarrhoea, nausea, dysesthesia, dyspnoea, myalgia, cognitive impairment, increased weight, cough, vomiting, pyrexia, arthralgia and vision disorders.
  - The most frequent serious adverse reactions (≥ 2%) were pneumonia, dyspnoea, pleural effusion, sepsis, pulmonary embolism, respiratory failure, and pyrexia.
  - Grade 3 or 4 adverse reactions occurred in 60% of patients.

More information on TRK inhibitors is provided in Module 1 of the *NTRK* gene fusions ESMO OncologyPRO portal.

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Abbreviations

ALT	Alanine transaminase
ANVISA	Agência Nacional de Vigilância Sanitária (Brazilian Health Regulatory Agency
AST	Aspartate transaminase
ATP	Adenosine triphosphate
BDNF	Brain-derived neurotrophic factor
CD34	Cluster of differentiation 34
CI	Confidence interval
DNA	Deoxyribonucleic acid
ESMO	European Society for Medical Oncology
FDA	Food and Drug Administration
FISH	Fluorescence *in situ* hybridization
GIST	Gastrointestinal stromal tumour
IDH1	Isocitrate dehydrogenase 1
IHC	Immunohistochemistry
MAPK	Mitogen-activated protein kinase
MASC	Mammary analogue secretory carcinoma
MSI	Microsatellite instability
NDA	New drug application
NGF	Nerve growth factor
NGS	Next-generation sequencing
NT-3	Neurotrophin 3
NT-4/5	Neurotrophin 4/5
NTRK	Neurotrophic tyrosine receptor kinase
PKC	Protein kinase C
PI3K	Phosphoinositide 3-kinase
RNA	Ribonucleic acid
RT-PCR	Reverse-transcriptase polymerase chain reaction
TK	Tyrosine kinase

Glossary

Term	Definition
FISH	Also known as fluorescence *in situ* hybridisation, it detects gene rearrangements in DNA that may generate a fusion transcript
Fusion protein	A protein created by the joining of two or more genes that originally coded for separate proteins
High-prevalence tumours	Tumours with a high likelihood of *NTRK* gene rearrangements. These include tumours with certain histologies or specific molecular subgroups, such as secretory carcinoma, infantile fibrosarcoma, pan-negative lung adenocarcinoma, wild-type and MSI-high colorectal carcinomas, IDH1 wild-type gliomas, pan-negative GIST and CD34-positive fibrosarcoma of soft tissue and bone.
IHC	Also known as immunohistochemistry, it detects protein expression which may be attributable to a fusion event
Tyrosine kinase domain	A structurally conserved region of protein kinases that contains the catalytic function of these enzymes
Low-prevalence tumours	Tumours with a low likelihood of *NTRK* gene rearrangements
NGS	Also known as next-generation sequencing, it detects known and novel fusions with breakpoints in DNA or RNA
NTRK gene	Gene coding for the neurotrophic tyrosine receptor kinase
NTRK gene fusions	Targetable driver genetic alterations that code for aberrant fusion proteins and drive tumourigenesis; these are formed by joining of the 3' region of the *NTRK* gene, including the kinase domain, with the 5' region of a different gene (i.e. the fusion partner) by intra- or inter-chromosomal rearrangement
RT-PCR	Also known as reverse-transcriptase polymerase chain reaction, it detects known fusion transcripts in RNA
TRK fusion proteins	The protein product of *NTRK* gene fusions, these proteins are constitutively active and result in cell growth, proliferation, and survival pathway activation
TRK inhibitors	A type of tyrosine kinase inhibitor and a type of targeted therapy against TRK fusion proteins
Tumour-agnostic treatment	A drug treatment that is used to treat any kind of cancer, regardless of where in the body or what tissue it initiated from
Tyrosine kinase	An enzyme that can transfer a phosphate group from ATP to a protein in a cell, it plays a critical activation and deactivation role in many cellular functions