NTRK connect®

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PRACTICAL GUIDANCE ON USE OF IMMUNOHISTOCHEMISTRY FOR THE DETECTION OF TRK FUSION-POSITIVE CANCER

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ENTRECTINIB & LAROTRECTINIB: TRK INHIBITORS

- The discovery of *NTRK* fusions led to the recent development of therapeutic agents that inhibit TRK fusion proteins
- Two TRK inhibitors are approved by the US Food and Drug Administration for use in patients with unresectable or metastatic *NTRK* fusion-positive cancers, agnostic of tumour type

ENTRECTINIB

INDICATION FOR USE: (extracted from the USPI)

1.2 *NTRK* Gene Fusion-Positive Solid Tumors

Indicated for the treatment of adult and pediatric patients 12 years of age and older with solid tumors that:

- have a neurotrophic tyrosine receptor kinase (*NTRK*) gene fusion without a known acquired resistance mutation,
- are metastatic or where surgical resection is likely to result in severe morbidity, and
- have either progressed following treatment or have no satisfactory alternative therapy.

This indication is approved under accelerated approval based on tumor response rate and durability of response *[see Clinical Studies (14.2)]*. Continued approval for this indication may be contingent upon verification and description of clinical benefit in the confirmatory trials.

LAROTRECTINIB

INDICATION FOR USE: (extracted from the USPI)

Indicated for the treatment of adult and pediatric patients with solid tumors that:

- have a neurotrophic receptor tyrosine kinase (*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.

Select patients for therapy based on an FDA-approved test *[see Dosage and Administration (2.1)]*.

This indication is approved under accelerated approval based on overall response rate and duration of response *[see Clinical Studies (14)]*. Continued approval for this indication may be contingent upon verification and description of clinical benefit in confirmatory trials.

NTRK, Neurotrophic Tyrosine Receptor Kinase; TRK, tropomyosin receptor kinase; USPI, United-States product information

USPI Larotrectinib dated 25 March 2021, USPI entrectinib dated 5 November 2021 (source: https://www.accessdata.fda.gov/scripts/cder/daf/index.cfm)

ADVANTAGES AND DISADVANTAGES OF IHC

Advantages of IHC for Testing for *NTRK* Fusions

- Widely available
- Relatively inexpensive, with a rapid turnaround time
- Less dependent on tumour purity compared with other biomarker testing methodologies

Disadvantages of IHC for Testing for *NTRK* Fusions

- Only detects the TRK protein expression without distinction between wild-type TRK and TRK fusion protein. As a result, a confirmatory test is needed to confirm the presence of a *NTRK* gene fusion if a positive IHC (≥1% positive cell) is observed
- Cytoplasmic staining alone without nuclear, peri-nuclear, or membranous staining may be simple background staining. Confirmation of fusion by a second assay should be conducted as *NTRK* fusions are extremely rare

IHC, Immunohistochemistry; TRK, tropomyosin receptor kinase

PAN-TRK ANTIBODIES

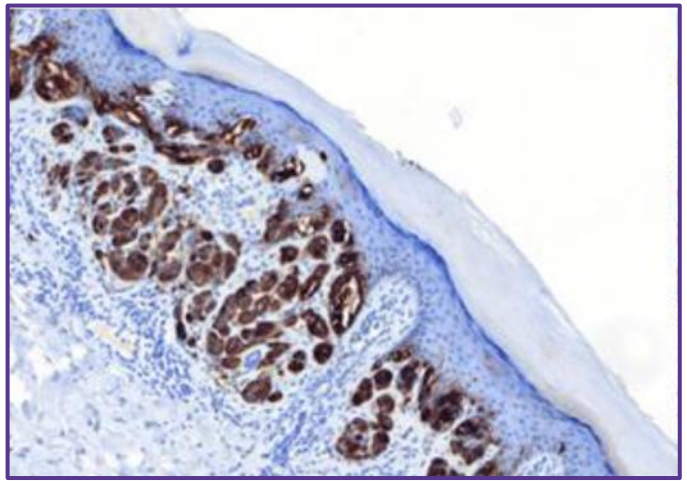
- Clone **EPR17341** is the most frequently used IHC antibody (purchased either as an *in vitro* diagnostic product or by itself):
  - Rabbit monoclonal antibody
  - Reactive to a homologous region of Trk-A, -B, and –C near the C terminus

- Clone **A7H6R** (purchased by itself)
  - Rabbit monoclonal antibody
  - Detects TrkA, TrkB and TrkC
  - May preferentially detect TrkA over TrkB, and TrkB over TrkC

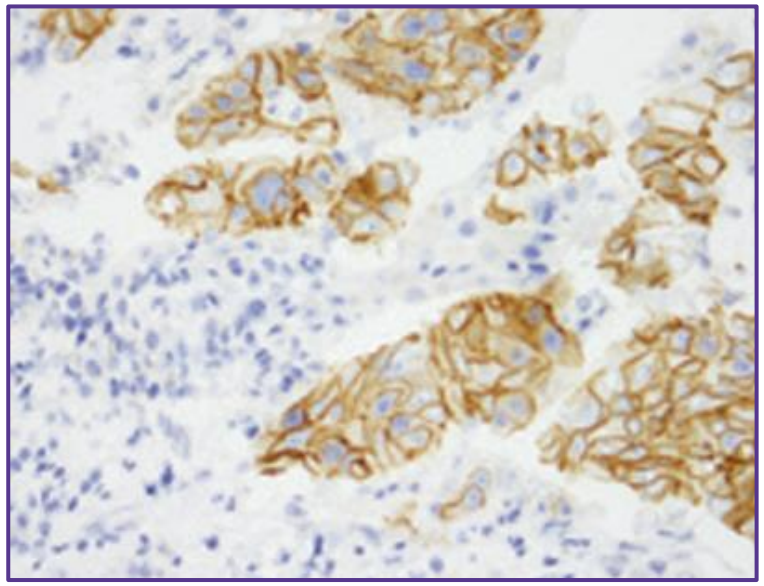
- When an other solution than the IVD is chosen a validation for clinical use within a lab is mandatory, as they are laboratory-developed tests

IHC, Immunohistochemistry; IVD, *In vitro* diagnostic; Trk, tropomyosin receptor kinase

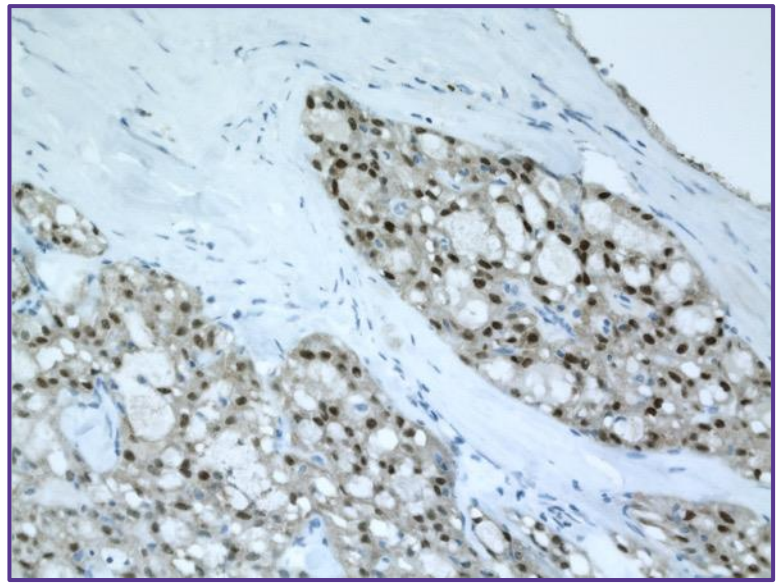
DIFFERENT PATTERNS OF TRK IHC STAINING



Cytoplasmic staining
EPR17341
Spitz tumour
NTRK1 fusion



Membranous staining
EPR17341 Intrahepatic cholangiocarcinoma *PLEKHA6-NTRK1* fusion



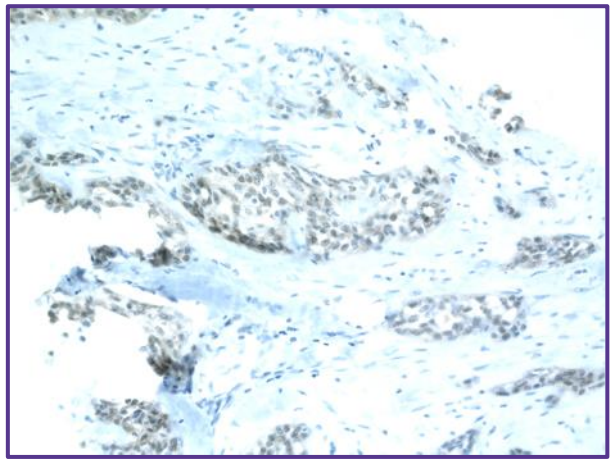
Nuclear staining
EPR17341
Salivary gland tumour
ETV6-NTRK3 fusion

IHC, Immunohistochemistry; NTRK, Neurotrophic Tyrosine Receptor Kinase; TRK, tropomyosin receptor kinase
Images courtesy Pr A Uguen (Spitz and Cholangiocarcinoma) and F Penault-Llorca (Salivary Gland Tumour)

EXQUISITE SENSITIVITY TO FIXATION

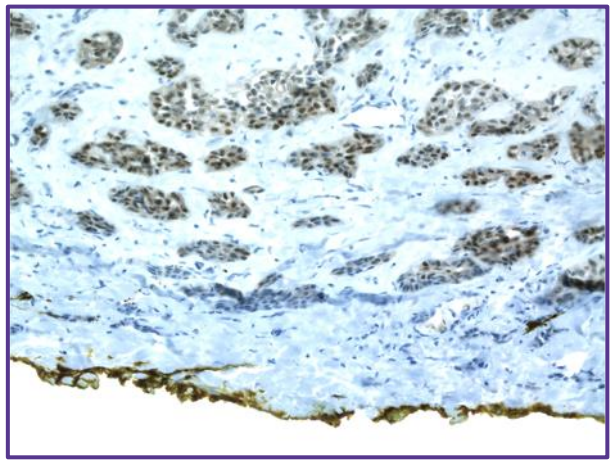
Secretory breast carcinoma

Centre of the tumour



Ventana EPR17341 prediluted

Border of the tumour



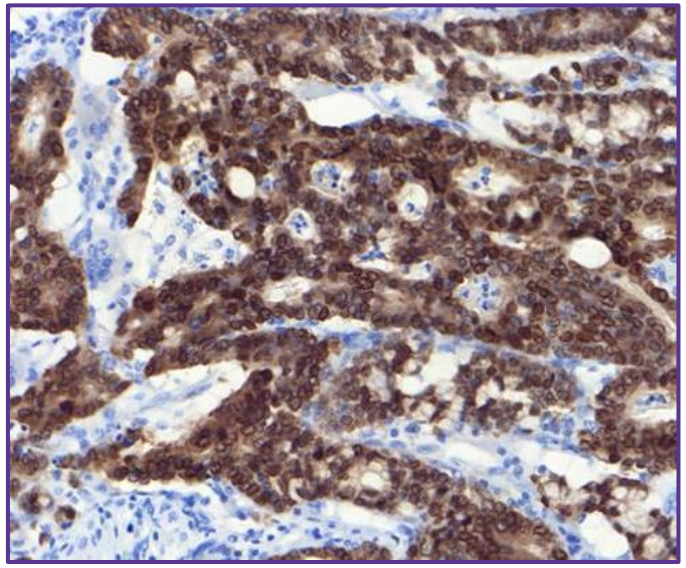
TRK staining in ≥1% of tumour cells is considered TRK fusion-positive

NTRK, Neurotrophic Tyrosine Receptor Kinase; TRK, tropomyosin receptor kinase

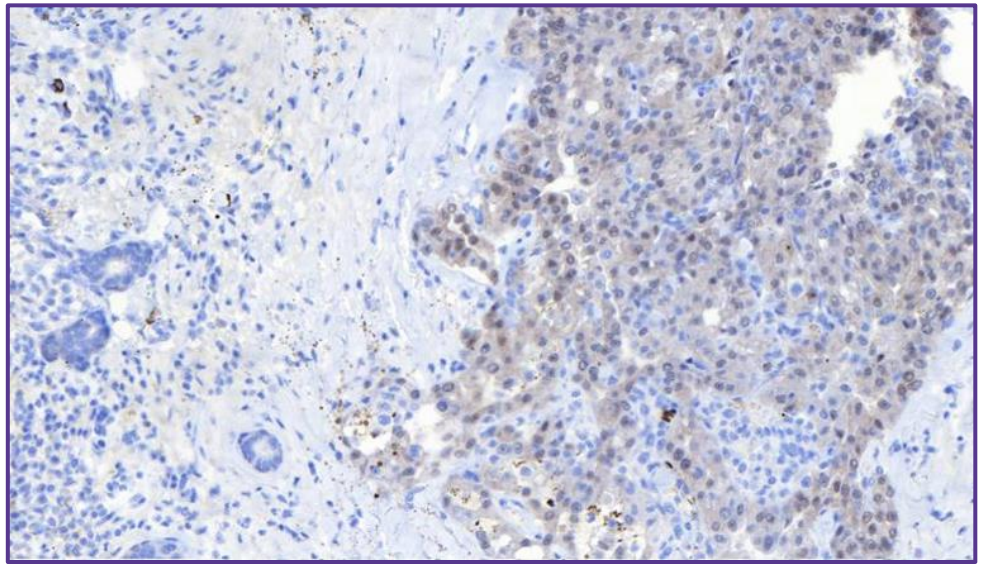
Images courtesy Pr F Penault-Llorca

PAN-TRK IHC HAS HIGH SENSITIVITY FOR *NTRK1-2* FUSIONS AND LOWER SENSITIVITY FOR *NTRK3*

- Pan-TRK IHC has demonstrated a sensitivity of:
  - 96.2% for *NTRK1* fusions
  - 100% for *NTRK2* fusions
  - 79.4% for *NTRK3* fusions



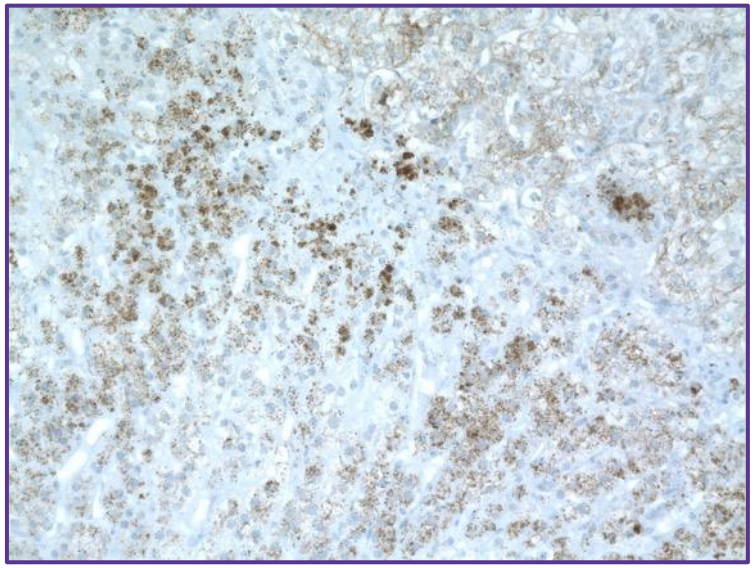
Colorectal carcinoma
NTRK1 rearrangement



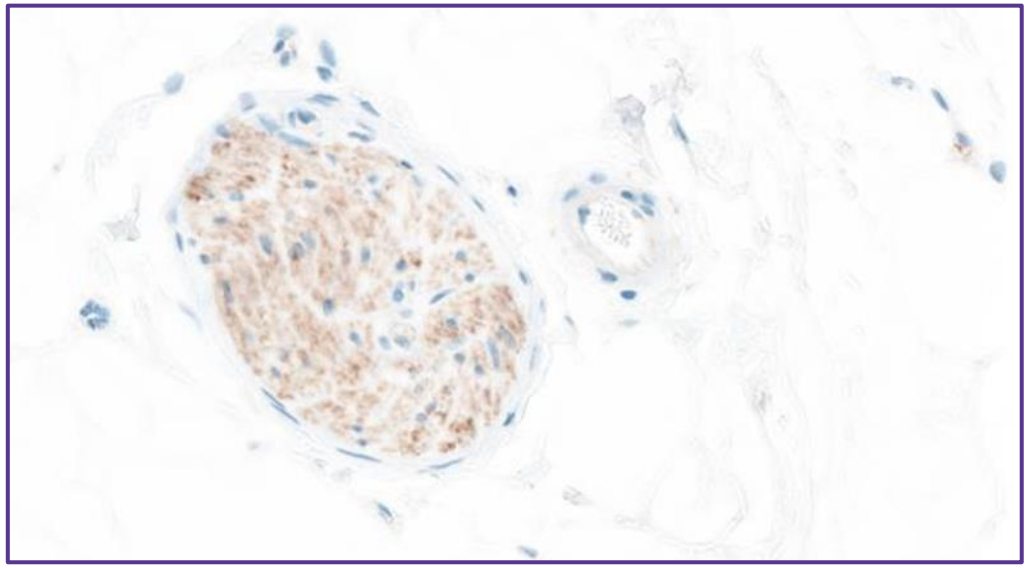
Colorectal carcinoma
NTRK3 rearrangement

IHC, Immunohistochemistry; NTRK, Neurotrophic Tyrosine Receptor Kinase; TRK, tropomyosin receptor kinase
Images courtesy Pr A Uguen

TRK PROTEINS ARE PHYSIOLOGICALLY EXPRESSED IN NON-NEOPLASTIC NEURAL AND SMOOTH-MUSCLE TISSUE



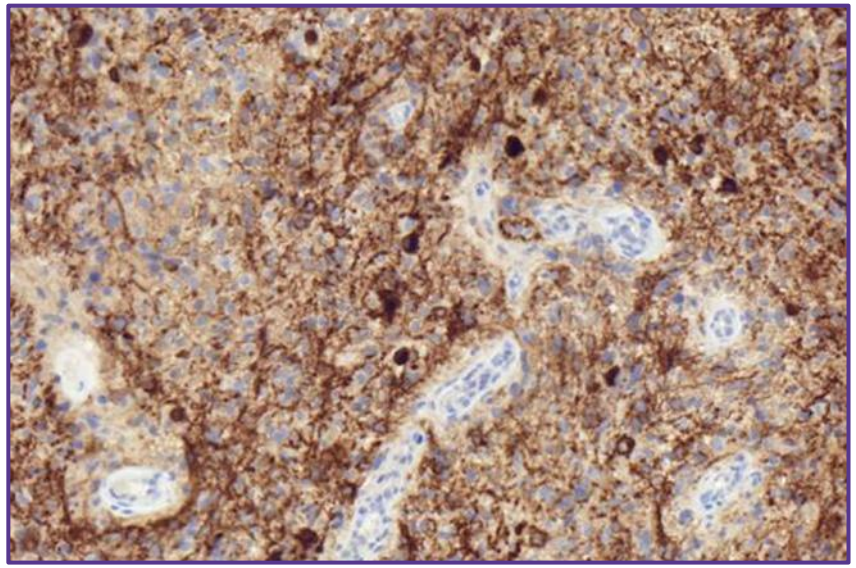
Adrenal gland cortex
Clone EPR17341



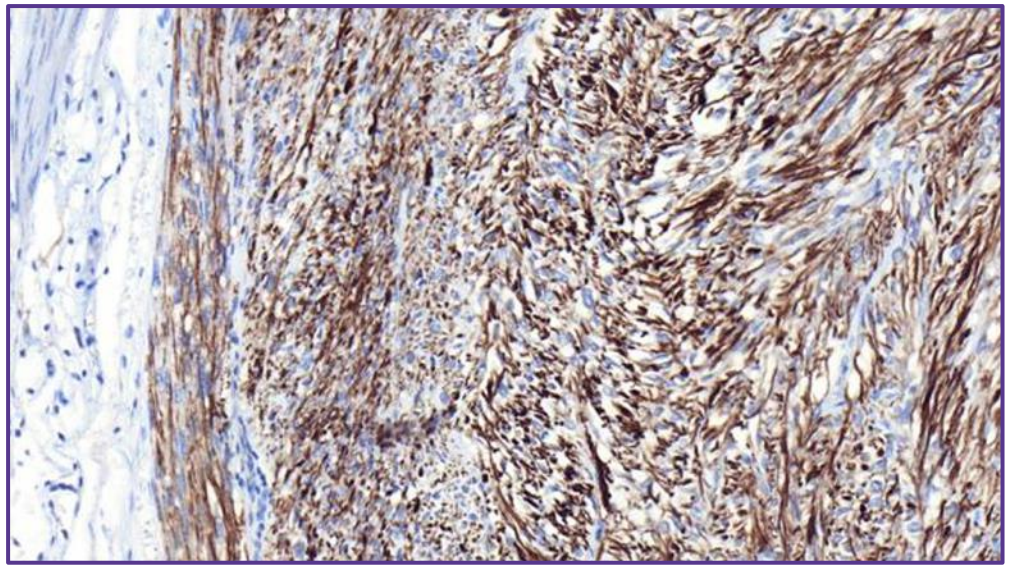
Ganglioneuronal cell
Clone EPR17341

TRK, tropomyosin receptor kinase
Images courtesy Pr A Uguen

OTHER METHODS OF *NTRK* FUSION TESTING SHOULD BE CONSIDERED FOR SARCOMAS, CNS TUMOURS, NEUROENDOCRINE TUMOURS AS FALSE POSITIVE STAINING MAY OCCUR



Glioblastoma
Clone EPR17341
Lack of *NTRK* fusion



GIST
Clone A7H6R
Lack of *NTRK* fusion

CNS, central nervous system; GIST, gastrointestinal stromal tumour; NTRK, Neurotrophic Tyrosine Receptor Kinase
Images courtesy Pr A Uguen

CONCLUSIONS

IHC is a good screening tool, but it is not enough

- **Confirmatory testing with nucleic acid-based analysis** should be performed in case of a positive IHC staining

Pathologists need to be aware of the limitations of IHC

- They have to keep in mind that a negative IHC result does not equal an absence of *NTRK* gene fusion

IHC, Immunohistochemistry; NTRK, Neurotrophic Tyrosine Receptor Kinase;

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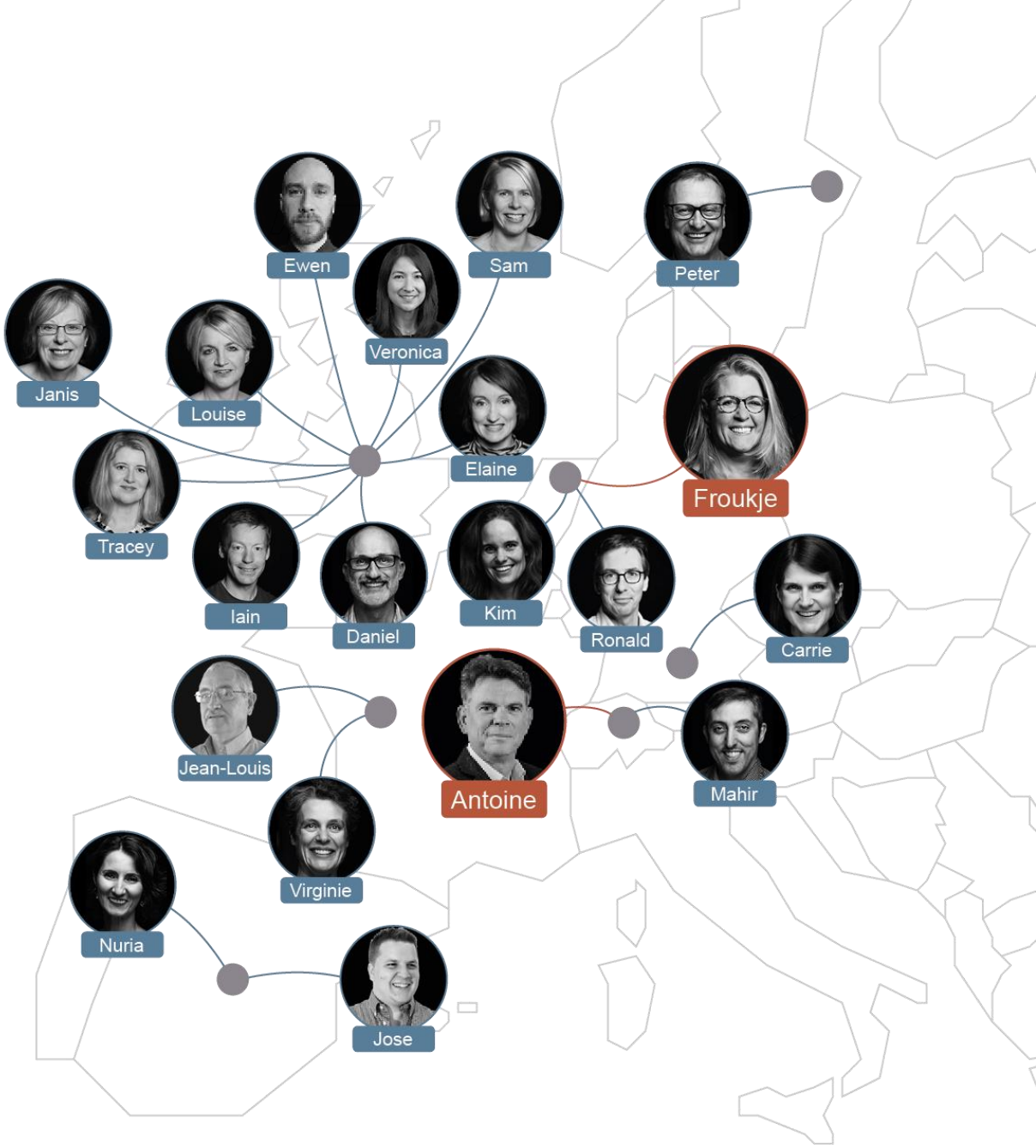

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