

Supplemental Materials

Table S1. Summary of Relevant Clinical Data for Included 30 Cases

| Study ID | Gender ¹ | Breed | Weight (kg) ² | Age (year) ² | Tumor type | Anatomic location | Met Score | LN metastasis ³ | Distant metastasis | Treatment | Survival time (days) |
|----------|---------------------|---------------------|--------------------------|-------------------------|------------|--|-----------|----------------------------|--------------------|----------------|----------------------|
| C01 | FS | Rottweiler | 34.0 | 2 | Accral | L front 5th digit | 1 | Y | Y | Sx | Lost to follow-up |
| C02 | FS | German Shepherd | 29.4 | 13 | Oral | Palatal to left maxillary canine (205) | 0 | N | N | Sx and Vaccine | 70 |
| C03 | FS | Labrador Retriever | 28.4 | 14 | Oral | R mandibular lingual mucosa between PM1 and M1 | 1 | N | N | Sx and RT | 288 |
| C04 | FS | Shar Pei | 28.1 | 15 | Oral | Lingual - R dorsal surface | 0 | Y - Suspected | Y | RT | 322 |
| C05 | FS | Shih Tzu | 5.6 | 9 | Cutaneous | dermal/ SQ L perianal mass | 0 | N | N | Sx and RT | 329 |
| C07 | FS | Giant Schnauzer | 42.5 | 11 | Cutaneous | R lateral antebrachium | 1 | N | N | None | Lost to follow-up |
| C08 | MN | Mixed | 37.6 | 8 | Oral | R lateral mandible around 408 | 2 | N | N | Sx and Vaccine | Lost to follow-up |
| C09 | FS | Labrador Retriever | 23.1 | 15 | Accral | L hind 3rd digit | 1 | N | Y | Sx and Vaccine | Lost to follow-up |
| C10 | FS | Miniature Schnauzer | 9.6 | 8 | Accral | R front 3rd digit | 2 | N | Y | Sx and Vaccine | 829 |

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|----------|---------------------|----------------------|--------------------------|-------------------------|------------|--|-----------|----------------------------|----------------------------|------------------------|----------------------|
| C11 | FS | Standard Poodle | 24.4 | 11 | Accral | L hind 2nd digit | 0 | N | N | Sx and Vaccine | Lost to follow-up |
| C15 | MN | Golden Retriever | 33.8 | 12 | Oral | Gingiva around 208 and 209 | 1 | N | Y - Suspected ⁴ | Sx and RT | 463 |
| C16 | MI | Labrador Retriever | 25.4 | 12 | Oral | Caudal to 109/110 | 0 | N | N | Sx and Vaccine | 657 |
| C17 | FS | German Shepherd | 28.0 | 10 | Cutaneous | R metacarpal pad | 1 | N | Y | Sx and Vaccine | Lost to follow-up |
| C18 | MN | Scottish Terrier | 12.3 | 11 | Oral | R upper lip mucosa | 1 | N | N | Sx | 1023 |
| C21 | MN | Basset Hound | 36.7 | 12 | Oral | R maxillary gingiva above 106 | 1 | N | N | None | 41 |
| C22 | MN | Golden Retriever | 40.8 | 7 | Oral | Sublingual | 0 | Y - Suspected | N | Sx and Vaccine | Lost to follow-up |
| C23 | FS | Gordon Setter | 14.7 | 9 | Oral | Mucocutaneous junction of R maxillary lip | 1 | N | N | Sx and Vaccine | Lost to follow-up |
| C24 | MN | Bernese Mountain Dog | 47.2 | 7 | Cutaneous | Ventral L thorax at the level of the 8th intercostal space | 0 | N | N | Sx, Vaccine, and Chemo | Lost to follow-up |
| C25 | FS | Gordon Setter | 29.0 | 11 | Oral | Gingiva on lingual aspect of 408 and 409 | 1 | Y - Suspected | Y | Sx, Vaccine, and Chemo | 193 |

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|----------|---------------------|--------------------|--------------------------|-------------------------|------------|---|-----------|----------------------------|----------------------------|------------------------|----------------------|
| C26 | FS | Saint Bernard | 68.8 | 11 | Oral | L mandibular lip | 0 | N | Y | Sx, Vaccine, and Chemo | 58 |
| C27 | FS | Labrador Retriever | 26.2 | 12 | Oral | L mandibular buccal gingiva | 0 | Y | N | Sx and RT | 434 |
| C28 | MN | Labrador Retriever | 39.6 | 13 | Oral | Rostral mand lingual region around incisors | 0 | Unknown | Unknown | None | Lost to follow-up |
| C29 | FS | Yorkshire Terrier | 3.2 | 13 | Oral | L mandible extending from 305 caudally | 0 | N | Y - Suspected ⁵ | Sx and RT | 130 |
| C30 | MN | Golden Retriever | 37.1 | 10 | Oral | dorsolateral pharynx near tonsil, along R mandibular ramus involving palate | 1 | N | Y | RT | 455 |
| C32 | MN | Pit Bull Terrier | 40.4 | 8 | Oral | R caudal maxilla | 1 | N | Y | Sx and RT | 427 |
| C33 | FS | German Shepherd | 41.6 | 13 | Oral | Caudal soft palate | 1 | N | Y - Suspected ⁶ | Sx and RT | 224 |
| C34 | MN | Labrador Retriever | 39.7 | 10 | Oral | R cd hard palate and soft palate in the region of 108 - 110 | 1 | N | N | Sx and RT | Lost to follow-up |
| C35 | MN | Giant Schnauzer | Unknown | 4 | Accral | R front digit 3 | 1 | Unknown | Y | Sx and Vaccine | 290 |

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|----------|---------------------|-----------------|--------------------------|-------------------------|------------|---------------------------------------|-----------|----------------------------|--------------------|----------------|----------------------|
| C37 | FS | German Shepherd | 39.9 | 8 | Cutaneous | L pinna - marginal cutaneous flap | 1 | Unknown | Unknown | Sx | Alive |
| C38 | FS | Cocker Spaniel | 7.1 | 9 | Oral | L mandibular gingiva near 305 and 306 | 2 | Unknown | Unknown | Sx and Vaccine | Alive |

1. FS = female spayed; MN = male neutered; MI = male intact

2. Weight and age at time of diagnosis

3. Suspected metastasis from LN palpation without cytologic support

4. Lytic bone lesion on mid-diaphysis of L radius on radiographs

5. Cutaneous melanoma on punch biopsy

6. Two round soft tissue opacities on thoracic radiographs may represent pulmonary metastasis or superimposition of structure

Table S2. Treatments elected among the 30 cases reviewed

| Treatment | No. of Cases | % of Total Cases |
|------------------------------------|--------------|------------------|
| Surgery and vaccine | 11 | 36.70% |
| Surgery and radiation therapy | 8 | 26.70% |
| Surgery only | 3 | 10.00% |
| Surgery, vaccine, and chemotherapy | 3 | 10.00% |
| No treatment | 3 | 10.00% |
| Radiation therapy only | 2 | 6.70% |

Table S3. Univariate analysis of histologic features and Met scores for all canine melanomas

| Histologic Feature | Values | Overall Met scores | | | |
|--|--------|--------------------|-----|-----|---------|
| | | 0 | 1 | 2 | p-value |
| Degree of pigmentation (count) | <50% | 6 | 11 | 2 | 0.52 |
| | >50% | 5 | 5 | 1 | |
| Presence of ulceration (count) | N | 4 | 3 | 1 | 0.57 |
| | Y | 7 | 13 | 2 | |
| Presence of necrosis (count) | N | 8 | 13 | 2 | 0.92 |
| | Y | 3 | 3 | 1 | |
| Presence of deep inflammation (count) ¹ | N | 6 | 6 | 1 | 0.41 |
| | Y | 4 | 6 | 2 | |
| Median mitotic index ² | -- | 3 | 3.5 | 2 | 0.95 |
| Median minimum Breslow thickness (mm) | -- | 10 | 4.4 | 3.7 | 0.79 |

1. 5 cases (4 orals and 1 acral) were not able to be classified due to lack of inflammation or tumor extension to tissue margin

2. Mitotic index = mitotic count per 10 high power fields

Table S4. Univariate analysis of histologic features and Met scores for oral canine melanomas

| Histologic Feature | Values | Oral melanoma Met scores | | | |
|--|--------|--------------------------|-----|-----|---------|
| | | 0 | 1 | 2 | p-value |
| Degree of pigmentation (count) | <50% | 4 | 7 | 1 | 0.67 |
| | >50% | 4 | 3 | 1 | |
| Presence of ulceration (count) | N | 3 | 0 | 1 | 0.48 |
| | Y | 5 | 10 | 1 | |
| Presence of necrosis (count) | N | 7 | 7 | 2 | 0.86 |
| | Y | 1 | 3 | 0 | |
| Presence of deep inflammation (count) ¹ | N | 5 | 2 | 1 | 0.27 |
| | Y | 2 | 5 | 1 | |
| Median mitotic index ² | -- | 2 | 4.5 | 1 | 0.6 |
| Median minimum Breslow thickness (mm) | -- | 9.5 | 4.7 | 3.4 | 0.86 |

1. 5 cases (4 orals and 1 acral) were not able to be classified due to lack of inflammation or tumor extension to tissue margin

2. Mitotic index = mitotic count per 10 high power fields

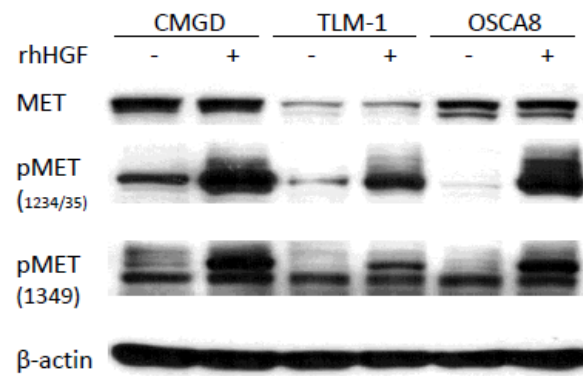


Figure S1. Recombinant human HGF (rhHGF) induces MET activation. CMGD-2 and TLM-1 are canine melanoma cell lines and OSCA-8 is a canine osteosarcoma cell line, used as positive control. All the experiments were performed after serum starvation and then stimulated with rhHGF (+) or untreated (-).

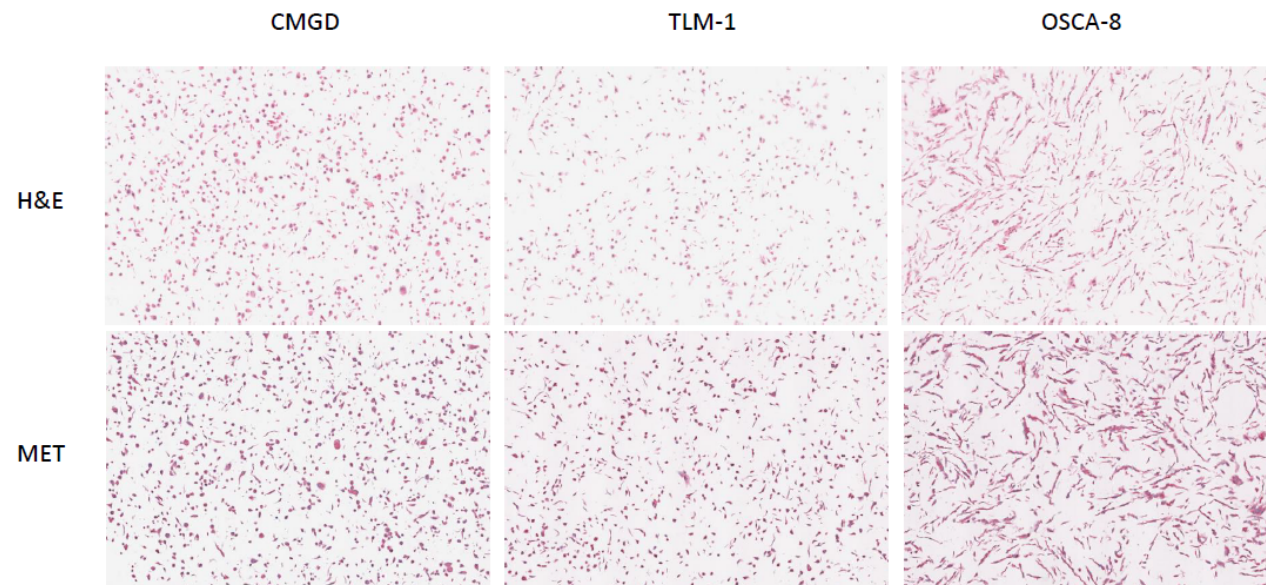


Figure S2. Confirmed immunopositivity with H&E staining and MET immunohistochemistry performed on cell pallets created from canine melanoma cell lines CMGD-2 and TLM-1 and canine osteosarcoma cell line OSCA-8 as positive control.